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MOISTURE DETERMINATIONS IN THE COMPARATIVE TESTING OF FORAGE CROPS FOR HAY YIELD[1]

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INTRODUCTION

Wherever forage crop varieties are tested for comparative yields of hay it is the general practice to sample the green material for moisture content at the time of cutting. When these tests are made up of small, randomized plots one of the common methods of sampling consists of taking one or two moisture samples per plot, usually one to two pounds of green material in size, drying to oven-dryness and calculating the percentage dry matter of each sample. On the basis of this determination the green yield per plot is converted to dry yield per plot which is later expressed in terms of tons or pounds of hay per acre. When in terms of hay yield per acre it is necessary to adjust the yield from absolute dry matter to include a certain amount of moisture, usually 12 to 15 per cent.

This procedure requires considerable equipment and a great deal of time. At many experimental stations proper ovens for drying large numbers of samples are often not available and thus some improvisations have to be made. At the Dominion Forage Crops Laboratory, Saskatoon, Saskatchewan, the electrically heated drying oven will hold only 45 samples at one time. Each lot of samples must remain in the oven a minimum of four hours, at a temperature of 212° F., in order to reach a constant weight. During the haying season, when as many as 2500 moisture samples are taken, it is necessary to have the oven operating for several weeks.

It would therefore seem advantageous to investigate the possibilities of eliminating or at least reducing the number of moisture determinations. Thus this study is an attempt to determine whether simplified moisture sampling techniques could be used without sacrificing the accuracy of yield comparisons.

LITERATURE REVIEW

A review of the literature shows that there are differences in opinions among various investigators as to the necessity of moisture determinations in comparative tests of forage crops for hay yields. McRostie and Hamilton (3) compared the relative forage yields of several plots of grass and clover mixtures, as determined from field cured hay, green yields, and absolute dry yields. From these comparisons it was concluded that both green yield weights and field cured hay weights were unreliable, with green yields

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being the least accurate of all the methods. They stated that immediate drying of shrinkage samples appeared to offer the most accurate criterion for comparative tests. In studying the number of shrinkage samples per plot necessary to give accurate results they found that more than 2-pound samples per plot were not warranted.

Weihing (7), from a study of the dry matter content in alfalfa, found that strains and replicates could vary significantly in percentage dry matter, for any one date of cutting. From this study Weihing concluded that alfalfa forage yields based on green yields were inaccurate. In a comparison between air-dried and oven-dried samples it was found that samples that had been air-dried under cover were nearly as accurate as those based on oven-dry weights. He also stated that for a comparison between cuttings or between years air-dry forage yields should be reduced to a definite percentage of dry matter.

In contrast to the above investigations, Wilkins and Westover (9) studied the moisture content of Turkestan and Grimm alfalfa and found that the difference in water content of the two varieties was so slight that the yield data could be based on the green weights. The average water content from 19 comparisons of the two varieties was 72.2 per cent for Turkestan and 72.1 per cent for Grimm. On the basis of these results green yields were used throughout the remainder of their study, which was a comparison of Turkestan alfalfa and Grimm alfalfa on wilt-infected soils.

Wilkins and Hyland (8) made a rather extensive study of moisture sampling in alfalfa and red clover. They found that, although the water content of the alfalfa forage varied with the location of the plots, the differences were so small that yield determinations would have been essentially as accurate on a green weight basis without sampling. The results for red clover were found to be similar to those for alfalfa.

In a study to determine the number of samples required to measure accurately the water content of alfalfa and red clover forage, Wilkins and Hyland (8) found that either two or three samples per plot were required. They also found that different sizes of samples from 1 to 10 pounds gave similar results. In one of their ten tests the 1-pound samples were found to be inadequate.

Wilkins and Hyland (8) and Willard (10) studied the moisture content of forage at different times of day. Wilkins and Hyland found that alfalfa forage was approximately 2.25 per cent lower in water content at 1.30 p.m. and at 5.00 p.m. than at 8.00 a.m., and that red clover was about 3.75 per cent lower than at 8.00 a.m. when similar comparisons were made. Willard obtained somewhat similar results in that he found that the variation in moisture content, after the dew was off, was very seldom greater than 3 per cent, in any of the crops studied, and as such was not sufficient to warrant concern over the time of day of cutting.

MATERIAL AND METHODS

Yield data from previous years were used for the first part of this study. These data consisted of green yields, dry matter percentages, and dry yields of most of the comparative tests conducted at the Dominion Forage Crops Laboratory, Saskatoon during the years 1940 and 1941.

This period was chosen because moisture sampling at that time was on the basis of one 1.5-pound sample per plot. The tests chosen for this study consisted of crested wheat grass strain tests, brome grass strain tests, tests which included several grass species, alfalfa strain and variety tests, and tests of sweet clover strains, varieties, and species. Grass and legume mixtures were not included because it was felt that the differences between dry matter content of the grass and of the legumes were too large, and mixtures were not sufficiently uniform for them to be included in a study of this kind.

The yield data from each test were analysed using Fisher's Analysis of Variance as described by Patterson (4). The variance analysis of dry yield data, green yield data and dry matter percentage data were all determined for each test and comparisons were made.

Sampling Study

In the summer of 1946 a moisture sampling study was conducted to evaluate and compare several methods of moisture sampling. The samples taken consisted of two 1.5-pound samples, two 0.75-pound samples and three 0.5-pound samples from each plot. A minimum green yield of 6 pounds per plot was therefore required. Due to the dry conditions that existed during the spring and summer of 1946, resulting in low productivity of most of the forage plots, it was necessary to confine the study to three varietal tests. These included a test of seven grass species, a sweet clover variety test, and a brome grass strain test. In the latter case the number of 1.5-pound samples had to be reduced to one instead of two per plot.

Description of Variety Tests Used in the Sampling Study

The first test, seeded in 1945, was a comparison of seven grass species arranged in a randomized block design with six replicates. The grass species included were: *Elymus virginicus*, *Elymus canadensis: Agropyron elongatum*, *Agropyron glaucum*, *Agropyron desertorum*, *Agropyron cristatum*, and *Bromus inermis*. The plots were 20 feet long by 6 feet wide, each consisting of 13 rows spaced 6 inches apart. There was a 1-foot pass between plots and a 2.5-foot pass at the ends of the plots.

The second test, seeded in 1945, originally consisted of 14 varieties of sweet clover in a randomized block arrangement with six replicates. Due to poor stands obtained only six of the varieties were harvested. Plots were 20 feet long by 6 feet wide, each consisting of 13 rows spaced 6 inches apart. There was a 1-foot pass between the plots and a 2.5-foot pass at the ends of the plots.

The third was a test of brome grass strains from uniform grass nurseries, also seeded in 1945. This test consisted of 16 different strains of *Bromus inermis*, eleven of which were from the United States Department of Agriculture, three were other American strains, and two were Saskatchewan strains, arranged in a quadruplicate lattice design. Each plot was 15 feet long by 6 feet wide and consisted of six rows spaced 1 foot apart. There were no passes between plots but there was a 1.5-foot pass at the ends of the plots. For the purpose of this study this test was treated as a randomized block.

Sampling Procedure

Before harvesting the plots for hay 1 foot was trimmed off each end of each plot to eliminate border effect. Thus for a 20-foot plot only the remaining 18 feet were used in determining the hay yield. Cutting of the plots was accomplished by means of a small power mower with a 42-inch cutting bar with a pan attached, to collect the hay as it was cut. Only one swath was taken from each plot and this was down the centre of the plot. The cut material was rolled in a cotton sheet and taken immediately to be weighed and the net green weight recorded. The samples for moisture determinations were then weighed without delay and placed in Kraft paper bags. All of the moisture samples were kept separate and numbered according to plot, size of sample, and order of weighing.

All weights were taken on a set of Fairbanks-Morse combination pan and platform scales with an enlarged pan. The beam of the scale was counterbalanced to offset the weight of the pan and the weight of the cloth used to hold the sample. Thus the reading on the beam gave the net weight of the green material to the nearest quarter ounce.

From the field the moisture samples were taken to the laboratory where they were removed from the paper bags and placed unchopped in drying trays, which in turn were placed in the electrically heated oven. The thermostatically controlled oven, similar to that described by McRostie and Hamilton (2), was heated to 212° F. and kept at that temperature until the drying was completed. Before being allowed to cool, the individual samples were weighed, including the drying tray, on a Dayton balance. This balance records weights to the nearest sixteenth of an ounce. The weight of the tray in each case was then deducted to give the absolute dry weight of the sample. From these data the dry matter percentage for each sample was calculated.

The data thus taken provided a comparison of three sizes of moisture samples and also allowed several shorter sampling methods to be compared with the standard method of one 1.5-pound sample per plot. The sampling methods used will be described as they are presented in the section dealing with experimental results.

EXPERIMENTAL RESULTS

The Necessity of Moisture Samples

A summary of the green and dry yield data of the comparative tests of 1940 and 1941, together with the F values and significant differences from the variance analyses of these data appear in Table 1. For each test in Table 1 the strains have been arranged in descending order of dry weight yield, the highest yielding strain in each case being designated with the letter A. The corresponding green yield for each strain appears below the dry yield. Therefore when the green yields are not in descending order it means that a change in the ranking of the varieties has occurred due to the effect of moisture sampling.

Test number 1, Table 1, is the 1940 data from an alfalfa variety test which included six varieties of *Medicago media*. It is seen that the F value from the green weight analysis is very similar to that of the dry weight

analysis and neither one is significant. The ranking of the varieties on the green weight basis is A, B, C, E, D, F whereas the ranking on the dry weight basis is A, B, C, D, E, F. The yield differences between varieties D and E are very small and since there are no significant differences between varieties then this change in the ranking could be considered as being due to a chance variation.

Test number 1A is the same test as number 1 but the data is for the year 1941. Here again it is seen that the F values for strains from the variance analyses of the green and dry yields are very similar and both are non-significant. The ranking of the strains is the same for both dry and green yields.

Tests 3, 5, 6, 6A, 7, 7A and 8 all give the same type of results as the tests discussed above, that is, very similar non-significant F values for strains for both dry and green weight analyses, and little or no change in the ranking of the strains. Any changes occurring in the ranking of the strains in these tests must be relatively unimportant because the strains do not differ significantly in yield.

Test number 2 is the 1940 data from an alfalfa variety test which contained five varieties of *Medicago media*. The F values in this test are slightly different, with that for dry yields being 2.94 and significant at the 5 per cent level and that for green yields being 2.14 and not significant. The F value at the 5 per cent level of significance is 2.87 for $n_1 = 4$ and $n_2 = 20$ degrees of freedom. The ranking of the varieties is the same for both dry and green yields. Thus the indication in this test is that dry yields gave a slightly more efficient test than green yields; however, the F value for dry yield analysis is so close to the 5 per cent level of significance that for most practical purposes it would not be taken too literally. Test 2A is the 1941 data of the same set of plots as for Test 2. The green yield F value is very close to being significant at the 5 per cent level and that for dry yields is significant. The ranking of the varieties is unchanged. It would thus appear that the reasonably small increase in efficiency in these two tests does not seem to justify the additional time and expense involved in the taking of moisture samples.

In Test 4 there are four strains of *Medicago media* and one strain of *Medicago falcata*. The F values for green and dry weight analyses are both significant and very similar. As in Test 3 the F value for green weights is slightly higher than the F value for dry weights. The order of varieties is unchanged. Thus the data indicate that for these two tests moisture sampling tended to increase the error variance rather than reduce it. When considering significant differences between the strains in test number 4 it is seen that the differences in average yield between strain A and strains C, D, and E are significant for both dry and green yields. Also the difference between strains B and E for green yields is significant but for dry yields it is non-significant. Since the 5 per cent level of significances is an arbitrary value such differences between significance and non-significance, as shown between strains B and E, would hardly be taken as conclusive.

Test 5A is another case where the F value for dry weights was significant at the 5 per cent level while the F value for green weights was not

TABLE 1.—SUMMARY OF THE DRY AND GREEN YIELD DATA, WITH F VALUES AND SIGNIFICANT DIFFERENCES, FOR THE COMPARATIVE TESTS CONDUCTED DURING THE PERIOD 1940-1941

Test No.	Material tested	Year	Yield data basis	Average yield of strains in pounds per plot												No. of reps.	F value for strains	L.D.S. between strains†
				A	B	C	D	E	F	G	H	I	J	K	L			
1	Alfalfa	1940	Dry	6.64	4.50	4.28	4.25	4.09	3.66							4	2.47	—
			Green	18.44	12.50	12.13	11.69	11.81	9.69							4	2.33	—
1A	Alfalfa	1941	Dry	3.63	2.80	2.36	2.32	2.13	2.04							4	2.06	—
			Green	9.26	6.76	6.01	5.63	5.13	5.13							4	1.88	—
2	Alfalfa	1940	Dry	1.64	1.60	1.29	1.25	1.19								6	2.94*	0.35
			Green	4.03	4.01	3.32	3.09	2.98								6	2.14	—
2A	Alfalfa	1941	Dry	1.66	1.48	1.23	1.19	1.08								6	3.38*	0.38
			Green	3.71	3.39	2.78	2.51	2.27								6	2.69	—
3	Alfalfa	1941	Dry	2.28	2.27	2.09	1.97	1.85	1.82							4	0.87	—
			Green	4.75	4.90	4.36	4.06	3.85	3.54							4	1.34	—
4	Alfalfa	1941	Dry	3.15	2.58	2.15	2.13	1.94								6	4.04*	0.71
			Green	8.10	6.94	6.18	5.32	5.07								6	4.22*	1.78
5	Brome grass	1940	Dry	2.17	1.95	1.88	1.84									6	0.23	—
			Green	6.66	4.31	4.10	3.92									6	0.15	—
5A	Brome grass	1941	Dry	2.78	2.33	2.33	1.82									6	3.33*	0.64
			Green	4.66	3.98	4.23	3.05									6	3.26	—
6	Crested wheat grass	1940	Dry	7.53	6.40	6.22	5.66	5.52	5.33							4	0.70	—
			Green	14.81	12.19	12.44	10.94	10.81	10.25							4	0.83	—
6A	Crested wheat grass	1941	Dry	4.97	4.53	4.47	4.47	4.05	3.64							4	0.59	—
			Green	8.47	7.30	7.61	8.28	6.89	6.64							4	0.51	—
7	Crested wheat grass	1940	Dry	10.02	8.76	7.60	7.60	7.34	7.08							4	1.21	—
			Green	16.94	13.59	12.63	13.25	12.19	11.94							4	1.39	—
7A	Crested wheat grass	1941	Dry	4.48	4.01	3.49	3.35	3.30	3.02							4	0.92	—
			Green	7.72	6.16	5.54	5.13	5.50	4.69							4	1.24	—
8	Crested wheat grass	1941	Dry	2.46	2.24	2.20	2.06	2.03	1.22							6	2.05	—
			Green	4.60	4.02	3.93	3.77	3.59	2.33							6	1.81	—
9	Standard grasses	1940	Dry	5.80	5.67	5.10	4.73	4.11	4.09							4	1.67	—
			Green	11.50	11.38	10.69	9.44	10.06	9.06							4	1.00	—
10	Standard grasses	1940	Dry	3.34	2.58	2.46	2.40	1.59								6	3.21*	0.91
			Green	6.39	5.74	4.77	4.90	3.50								6	4.23*	1.56
10A	Standard grasses	1941	Dry	4.20	3.37	2.54	2.48	2.40								6	10.26**	0.97**
			Green	6.83	5.35	4.27	4.23	5.69								6	5.80**	1.81**
11	Sweet clover	1940	Dry	5.86	5.76	5.23	4.16	4.11	3.99	3.98	3.96					6	4.38**	1.60**
			Green	19.00	19.67	18.93	13.75	13.67	15.10	13.76	12.89					6	3.62**	5.77**
12	Sweet clover	1941	Dry	4.23	4.19	3.53	3.50	3.47	3.34	3.20	3.00	2.93				6	4.74**	0.61*
			Green	13.34	12.38	12.46	10.41	11.60	10.86	10.75	10.38	10.05				6	2.17*	2.22*
13	Sweet clover	1941	Dry	3.16	3.07	3.04	2.67	2.61	2.50	1.97	1.79	1.69	1.45	1.16	1.04	6	15.09**	0.74**
			Green	9.56	10.53	9.12	9.52	9.39	7.36	6.93	5.96	5.33	5.24	3.99	3.38	6	13.78**	2.47**

* Significant at the 5 per cent level. ** Significant at the 1 per cent level. † L.S.D. = Least significant diff[illegible]

quite significant. Again no clear distinction should be drawn between the two sets of analyses as the difference is extremely small. The order of strains changed very slightly but it was far from being a significant change.

All of the tests discussed up to this point could be classed as one-crop tests because only one crop has been included in each. Even though some of these tests included more than one species it is apparently quite evident that the differences in dry matter content between the species must be relatively small. It is also evident that the green weight analyses of these tests have been as good as the dry weight analyses; at least from a practical viewpoint the detailed work of moisture sampling has not been justified.

Test number 9 is a comparative test consisting of four strains of crested wheat grass, one strain of brome grass, and one strain of slender wheat grass. As can be seen in Table 1 the F values for strains for dry and green weights are again similar and non-significant. There is one change in the order of strains but this cannot be considered as significant.

Test number 10 consisted of *Elymus junceus*, *Bromus inermis* (Common), *Agropyron cristatum* (Fairway), *Agropyron trachycaulum* and *Agropyron desertorum*, five distinctly different grasses. The F values for green and dry weight analyses are both significant to the 5 per cent level and differ by 1.02, with that for green weights being the higher one. The ranking of strains changes from A, B, C, D, E for dry weights to A, B, D, C, E for green weights. On the dry weight basis the differences between strain A and strains D and E are significant; also the difference between B and E is significant. On the basis of the green weight analysis the difference between strain A and strains C and E is significant; and also the difference between B and E is significant. Strains C and D are not significantly different in either analysis. This, therefore, is a case where the use of green weights appears to have altered the significant relationship between certain of the strains.

The 1941 data of the same set of plots, Test 10A, shows even greater differences between green and dry weight analyses. In a comparison of the F values for strains it is seen that although they both surpass the 1 per cent level of significance the F value of the dry weight analysis is almost double what it is for the green weight analysis. This would indicate that by the use of moisture data the experimental error has been reduced and the actual differences between strains have become more pronounced, thus increasing the efficiency of the test. It is also seen that the ranking of the strains on the dry weight basis, A, B, C, D, E, differs considerably from the ranking on the green weight basis, A, E, B, C, D. Strain E has been changed from the lowest yielding strain to the second highest. With changes as great as these it is quite evident that for this particular test green weights for comparative purposes would not be reliable.

The sweet clover tests, numbers 11, 12, and 13, included several strains of *Melilotus alba*, *M. officinalis*, and *M. suaveolens*. These species differ in type of plant, time of flowering, percentage of leaf, and several other characters. In Table 1 it is seen that the F values for dry and green weight analyses differ quite considerably. The ranking of strains as determined by dry and green weights in each test differ rather markedly,

with many significant changes occurring in the relationships of the strains. For example, in Test 11 varieties A and B are both highly significantly different to varieties D, E, F, G, and H on the basis of dry weights. When using green weight data variety B differs significantly from varieties D, E, G and H but A is only significantly different to H.

Again it is obvious that green weights, as a basis for comparison of treatments in these tests, would be unreliable.

It thus becomes apparent that the data in Table 1 could be classified into two distinct groups, one-crop tests, as previously mentioned, and secondly, several crop tests. Some further evidence for this grouping is obtained from an examination of the dry matter percentage data from these tests, which appear in Table 2. In Table 2 the order of the strains for each test corresponds to the order of strains given in Table 1.

Of the thirteen one-crop tests, numbers 1 to 8, inclusive, there are only six cases in which the F value for strains is significant, and of these, three are significant at the 1 per cent point. In these tests, 4, 5A, and 7A, although the strains are highly significantly different the actual maximum differences in dry matter percentage are relatively small, less than 15 per cent of the lower range. It has already been pointed out that in these tests the green weight analysis gave essentially the same results as the dry weight analysis. In seven of the thirteen tests replicates are shown to be significantly different, and of these, three are significant at the 1 per cent point. Significant differences between replicates in the dry matter percentage appear to have very little effect on yield data. An example of how little these differences affect the yield data is given by Test number 1A where strain differences are not significant and replicates are highly significantly different. On referring back to Table 1 it is seen that the variance analysis of the green yields gave essentially the same results as the variance analysis of the yields after the dry matter percentages had been taken into consideration.

The six tests, numbers 9 to 13, inclusive, show somewhat different results. In every case strains are highly significantly different for dry matter percentage, with maximum differences between the strain averages generally being greater than for tests 1 to 8, inclusive. It is therefore to be expected that for this group of tests green weight analysis would give very different results from dry weight analysis.

Since the one-crop tests have such small differences in dry matter percentage between the strains, and since the green weight analysis appears to be as satisfactory as dry weight analysis, for comparative purposes, detailed moisture sampling would be unnecessary. However, the general practice of reporting forage yields is to report, at 12 to 15 per cent moisture, the pounds or tons of hay produced per acre. The most practical method of reducing green yields to dry yields and then back to the desired percentage moisture would be to take five or six moisture samples at random over the entire test, determine the average percentage dry matter, and use this average to change green weights to hay weights. The comparison of the hay yields on this basis would be exactly the same as the comparison on the basis of the green yields. By using this method the data become

TABLE 2.—SUMMARY OF THE DRY MATTER PERCENTAGE DATA, WITH F VALUES AND SIGNIFICANT DIFFERENCES, FOR THE COMPARATIVE TESTS CONDUCTED DURING THE PERIOD 1940-1941

Test No.	Material tested	Year	Treatment	Average per cent dry matter A	B	C	D	E	F	G	H	I	J	K	L	F values	L.S.D.†
1	Alfalfa	1940	Strains	36.0	35.8	35.4	36.5	35.5	37.9							1.21	—
			Reps.	36.7	36.5	36.3	35.2									0.85	—
1A	Alfalfa	1941	Strains	39.0	41.0	41.1	41.0	42.6	41.5							0.56	—
			Reps.	43.4	42.3	41.8	36.6									5.49**	5.35
2	Alfalfa	1940	Strains	41.2	40.4	38.7	41.3	40.5								1.16	—
			Reps.	41.8	41.5	41.4	41.3	39.2	37.2							2.94**	3.2
2A	Alfalfa	1941	Strains	46.3	44.4	45.0	47.6	47.9								0.99	—
			Reps.	51.5	46.4	46.4	45.7	44.1	43.4							2.86*	5.0
3	Alfalfa	1941	Strains	48.0	46.9	48.2	48.6	48.3	51.4							1.24	—
			Reps.	50.6	49.0	48.5	46.1									2.91	—
4	Alfalfa	1941	Strains	38.5	37.2	34.9	40.2	38.3								6.32**	3.1
			Reps.	39.9	39.5	36.8	36.8	37.4	36.4							3.19*	2.5
5	Brome grass	1940	Strains	48.9	45.3	46.0	46.9									3.58*	2.5
			Reps.	47.9	47.3	46.9	46.5	46.1	45.9							0.55	—
5A	Brome grass	1941	Strains	60.0	59.2	55.1	60.2									14.31**	2.6
			Reps.	62.4	60.5	58.2	57.6	57.2	55.9							9.47**	3.2
6	Crested wheat grass	1940	Strains	50.9	52.1	50.1	51.5	50.5	51.4							0.96	—
			Reps.	52.1	52.0	51.4	49.0									5.70**	2.5
6A	Crested wheat grass	1941	Strains	59.6	62.0	59.0	53.4	58.8	54.7							3.76*	5.0
			Reps.	59.2	58.3	57.1	57.0									0.62	—
7	Crested wheat grass	1940	Strains	59.0	63.1	59.4	57.0	59.4	58.9							3.51*	3.2
			Reps.	61.9	59.8	58.4	57.7									4.67*	3.7
7A	Crested wheat grass	1941	Strains	58.2	65.5	64.1	65.7	60.2	65.4							11.90**	3.9
			Reps.	64.5	63.9	62.9	61.5									3.04	—
8	Crested wheat grass	1941	Strains	53.7	56.2	56.8	54.8	56.0	52.1							1.50	—
			Reps.	56.7	56.5	55.5	54.4	53.7	52.9							1.12	—
9	Standard grasses	1940	Strains	49.8	49.5	47.8	48.4	40.7	44.5							4.72**	6.8
			Reps.	49.3	48.3	45.9	43.7									3.58*	4.0
10	Standard grasses	1940	Strains	52.2	45.1	51.6	48.8	45.2								41.26**	2.1
			Reps.	49.7	49.3	48.6	48.1	47.9	47.6							2.04	—
10A	Standard grasses	1941	Strains	61.6	63.3	60.0	60.2	41.2								147.87**	3.0
			Reps.	58.5	57.6	57.3	56.8	56.6	56.5							0.88	—
11	Sweet clover	1940	Strains	30.8	29.6	29.1	30.3	30.3	29.1	26.2	31.1					11.32**	1.8
			Reps.	30.6	30.3	30.1	29.6	28.4	28.2							6.50**	1.5
12	Sweet clover	1941	Strains	31.7	34.3	28.6	33.7	30.0	30.8	30.1	29.2	29.3				7.74**	2.8
			Reps.	32.6	31.3	30.6	30.6	30.4	30.0							2.00	—
13	Sweet clover	1941	Strains	33.2	29.3	33.4	28.0	28.1	34.1	28.5	30.0	32.7	28.0	28.7	28.7	14.16**	2.4
			Blocks	30.8	30.5	30.4	30.3	30.2	29.1							1.67	—
			Columns	31.0	30.7	30.5	30.4	29.5	29.1							2.78*	1.3

* Significant at the 5 per cent level. ** Significant at the 1 per cent level. † Least significant difference.

comparable with the yield data from other years and other stations. Moisture sampling therefore would not have been eliminated but the number of samples required would be greatly reduced.

The summary of the several-crop tests clearly indicates that where large differences in dry matter percentage occur between forage crop species, that are included in any one test, it is necessary to take moisture samples by some method that will bring out these differences.

SAMPLING STUDY

Test of Various Grass Species

A summary of the dry matter percentage data and the variance analysis of these data are presented in Tables 3 and 3a, respectively.

TABLE 3.—DRY MATTER PERCENTAGES, AVERAGE OF SIX REPLICATES, AS DETERMINED FROM DIFFERENT SIZES OF MOISTURE SAMPLES FOR A TEST OF SEVEN GRASS SPECIES

Grass species	Size of sample and sample number							Means of species
	0.5 pound			0.75 pound		1.5 pounds		
	1a	1b	1c	2a	2b	3a	3b	
Agropyron glaucum	51.6	50.5	50.1	49.4	49.9	50.8	50.6	50.4
Elymus virginicus	49.8	49.9	49.9	49.1	49.5	49.8	49.4	49.3
Agropyron desertorum	50.6	49.4	49.3	48.4	48.0	46.6	45.4	48.3
Elymus canadensis	47.3	46.9	47.4	46.4	47.6	47.3	47.1	47.1
Agropyron cristatum	48.2	47.4	47.6	46.7	46.4	44.3	45.4	46.6
Agropyron elongatum	43.1	41.6	43.9	41.8	42.5	42.0	42.2	42.4
Bromus inermis	36.0	35.3	34.9	33.6	33.4	31.8	32.0	33.9
Sample means	46.6	45.8	46.2	45.1	45.0	44.7	44.6	
Means of sample size	46.2			45.0		44.6		

Least significant difference between means of species, 1 per cent level = 2.4 per cent.
Least significant difference between means of sample size, 5 per cent level = 0.5 per cent.

TABLE 3a.—VARIANCE ANALYSIS OF THE DRY MATTER DATA OF THE TEST OF SEVEN GRASS SPECIES

Source of variation	Degrees of freedom	Sum of squares	Mean square	F. value
Species	6	8192.72	1365.45	86.59**
Replicates	5	107.76	21.55	1.37
Error (*a*)	30	473.09	15.77	
Plots	41	8773.57		
Between sample size	2	146.54	73.28	49.51**
Within sample size	4	16.20	4.05	2.74*
Species × between sample size	12	142.08	11.84	8.00**
Species × within sample size	24	45.79	1.91	1.29
Error (*b*)	210	311.35	1.48	
Total	293			

* Significant at the 5 per cent level.
** Significant at the 1 per cent level.

Table 3a shows that the grass species differ very significantly in dry matter content. It is seen in Table 3 that *Bromus inermis* produced the lowest average per cent dry matter, 33.9 per cent, and *Agropyron glaucum* produced the highest percentage with 50.4 per cent. In this test the replicates were not significantly different for dry matter percentage. The differences between the means of the different sizes of samples were highly significant. The means of the 0.75-pound samples and the 1.5-pound samples are very significantly lower than the mean of the 0.5-pound samples, even though these differences were extremely small. The general indication from these data is that as the size of the moisture sample decreased the percentage of dry matter increased. One plausible explanation of this peculiarity might be that the scale used for weighing the green samples may have had a small constant error, although the scale was thoroughly checked before each experiment. If this error was such that instead of obtaining the required weight of 1.5, 0.75, or 0.5 pounds of green material, the weight required plus an additional constant amount was obtained, then as the size of sample decreased the constant error would become proportionately larger. The extra amount of green material would thus cause the smaller samples to show a slightly higher dry matter percentage.

The variation within each size of sample was also greater in the 0.5-pound samples than in the larger samples. The only significant difference between samples within a size is between samples 1a and 1b. This difference is very small but it does tend to indicate that the 0.5-pound samples are not as reliable as the 1.5-pound samples. The significant interaction of species x between sample size is a further indication that the small samples are not as reliable as the larger samples. This significant interaction might possibly have been caused by small samples giving a lower percentage dry matter than the larger samples in some of the more leafy species. The interaction of species x within sample size is not significant. This would indicate that each size of sample was consistent in sampling any particular species.

The very low variability of this sampling test tends to exaggerate very small differences and make them seem important. In actual forage crop testing the variability, due to uncontrollable factors, is so much larger that these very small differences no longer seem important. The coefficient of variability for the sampling data is only 2.68 per cent, whereas on the actual yield data the coefficient of variability for this test is 13.78 per cent.

(a) Number of Samples Per Plot

The standard method of moisture sampling used at the Dominion Forage Crops Laboratory, Saskatoon, consists of taking one 1.5-pound sample from each plot in a test. It was desirable to ascertain the relative efficiency of this one sample per plot as compared to two or more samples per plot. Using a procedure given by Snedecor (5) and used by Torrie *et al.* (6), it is possible to estimate the relative efficiency of increasing both the number of samples per plot and the number of plot replications.

Torrie *et al.* pointed out that field experiments in which samples are taken to represent the whole plot have two sources of random variation, the sampling and experimental errors. The sampling error is the variance

between samples within a plot. Experimental error is made up of two sources of variation, the random variation of plots within a replicate and the sampling error. The random variation of plots within a replicate is designated as A and the variance of the mean of k samples about the mean of the plot is B/k. Thus the experimental error variance in terms of plot means is $A + \frac{B}{k}$, which in terms of individual samples would be $k\left(A + \frac{B}{k}\right) = kA + B$. The estimated variance of a species mean on a single plot basis, $V\bar{x}$, would be $V\bar{x} = \frac{kA + B}{kr}$, where k is the number of samples and r is the number of replicates.

The dry matter percentage data, as determined from each sample, were grouped according to size and a separate analysis was made of each group. The summary of these analyses appears in Table 4.

The experimental error, error (*a*) Table 4, is represented by k A + B which, in the case of 1.5-pound samples, is equal to 5.33. The sampling error, error (*b*) is represented by B and is equal to 0.83. The number of samples per plot, K, equals 2. Thus the value of A is 2.25. From these data $V\bar{x}$ can now be calculated.

$$V\bar{x} \text{ where } k = 2,\ r = 6, = \frac{kA + B}{kr} = \frac{2\,(2.25) + 0.83}{2 \times 6} = 0.444$$

$$V\bar{x} \text{ where } k = 1,\ r = 6, = \frac{1\,(2.25) + 0.83}{1 \times 6} = 0.513$$

$$\text{the relative precision factor in per cent} = \frac{0.513}{0.444} \times 100 = 116$$

TABLE 4.—SUMMARY OF THE VARIANCE ANALYSES OF EACH OF THE SAMPLE SIZES, FOR THE TEST OF SEVEN GRASS SPECIES

Variation due to	0.5-Pound samples		0.75-Pound samples		1.5-Pound samples	
	DF	Mean square	DF	Mean square	DF	Mean square
Species	6	532.93	6	378.75	6	477.45
Replicates	5	13.04	5	7.87	5	4.79
Error (*a*)	30	7.77	30	6.17	30	5.33
Main plots	41	—	41	—	41	—
Samples	2	7.03	1	0.006	1	0.15
Error (*b*)	82	1.79	41	1.27	41	0.83
Total	125	—	83	—	83	—

It is thus seen that two samples per plot increased the estimated sampling efficiency by 16 per cent. The coefficient of variability for sampling in the analysis shown in Table 4 for the 1.5-pound samples was 2.04 per cent. The dry yield data, as determined from using moisture sample 3b, showed a coefficient of variability of 13.02 per cent. It is quite

obvious that an increase of 16 per cent in the moisture sampling would affect the yield data very little. Thus, for practical purposes, one 1.5-pound moisture sample per plot would be essentially as good as two samples per plot.

The above procedure was also used to determine the relative efficiency of the different sizes of samples. The estimated variances of species means on a single plot basis, $V_{\bar{x}}$ values, and the relative precision factors, using different numbers of samples, were calculated for the three sizes of moisture samples, and are shown in Table 5.

TABLE 5.—THE ESTIMATED VARIANCES OF SPECIES MEANS AND RELATIVE PRECISION FACTORS FOR DIFFERENT NUMBERS OF SAMPLES PER PLOT WITH SIX REPLICATES, FOR DIFFERENT SIZES OF MOISTURE SAMPLES

Sample size (pounds)	Mean square		Estimated variance of a plot A	Estimated variance of a species mean and relative precision factor					
	Experimental Error kA + B	Sampling Error B		k = 1		k = 2		k = 3	
				$V_{\bar{x}}$	P.F.*	$V_{\bar{x}}$	P.F.	$V_{\bar{x}}$	P.F.
0.5	7.77	1.79	1.99	0.630	70	0.481	92	0.432	103
0.75	6.17	1.27	2.45	0.620	72	0.514	86	0.479	93
1.5	5.33	0.83	2.25	0.513	87	0.444	100	0.421	105

* P.F. = Precision factor or estimated efficiency in per cent.

The relative precision factors in Table 5 are expressed in terms of k = 2, r = 6, for 1.5-pound samples, as 100. It is seen that one 1.5-pound moisture sample per plot is a relatively more efficient sampling procedure than using one 0.5-pound sample or one 0.75-pound sample per plot. It is also noted that one 1.5-pound sample per plot gives approximately the same relative efficiency as two 0.75-pound samples per plot and only slightly less than two 0.5-pound samples per plot.

On the basis of the above data it is apparent that the use of one 1.5-pound sample per plot, as a standard method of sampling this six replicate test, is justified. The data further indicate that the moisture samples that were less than 1.5 pounds were more variable and hence not as reliable as the larger samples.

(b) Sampling Methods

It would be advantageous to have sampling methods that were shorter and less detailed, than the standard method of sampling, but which would give results that were essentially as accurate. For tests involving 6 replicates some suggested shorter methods are:

1. One 0.5-pound sample of green material taken from each plot. The samples from the first three replicates would be bulked for each species, and dried as one 1.5-pound sample. Similarly the three samples for each species for the last three replicates would be bulked and dried as another sample. The dry matter percentage as determined for each sample would then be used to calculate the dry yield for each particular species in its respective replicates. By using this method only one-third of the normal number of samples would be dried.

2. One 0.75-pound sample taken from each plot. The two samples, for each species, from replicates 1 and 2, would be bulked and dried as one sample. Similarly the samples from replicates 3 and 4, and 5 and 6 would be bulked and dried. The dry matter percentage would then be used to calculate the dry yield of the particular species in its respective replicates. This method would reduce the number of samples to be dried to one-half of the usual number.

3. One 1.5-pound sample taken from each plot in one replicate, chosen at random. The dry matter percentage for each species from this one replicate would then be applied to all replicates. This would reduce the number of samples to one-sixth of the normal number required.

4. One 1.5-pound sample from each plot in each of two replicates, chosen at random. The two determinations for each species would then be averaged and applied to all replicates. In this case only one-third of the normal number of samples would be required.

In order to obtain a comparison of these methods with the standard method the available data were arranged to conform, as closely as possible, to the suggested methods. The first two methods could not be made up as described because when carrying out the sampling study each sample was dried individually. Therefore, Method 1, as it appears in Table 6, was made up from a random selection of one 0.5-pound sample from each plot and then the average percentage dry matter determined for the first three replicates and for the last three replicates in each species. Similarly for Method 2 individual determinations were selected from each plot, at random, and averaged for each set of two replicates. In Methods 3 and 4 the dry yield data were determined from the dry matter percentages of sample 3b. Sample 3a was used as the check method; this also was chosen at random.

Table 6.—Summary of the dry yield data of the test of seven grass species, as determined by the different sampling methods, with the F values and least significant differences included

Grass species	Key to species	Average yield in pounds per plot METHODS				
		1	2	3	4	Check
Agropyron elongatum	A	6.23	6.20	6.06	6.26	6.15
Agropyron glaucum	B	5.99	5.99	5.78	6.01	6.07
Elymus canadensis	C	5.00	4.99	4.56	5.03	4.97
Agropyron desertorum	D	4.61	4.44	4.15	4.10	4.25
Agropyron cristatum	E	3.79	3.72	3.67	3.59	3.51
Bromus inermis	F	3.73	3.58	3.69	3.31	3.41
Elymus virginicus	G	2.73	2.66	2.63	2.75	2.74
F values		27.76**	33.16**	29.52**	34.18**	28.74**
Least significant difference 1 per cent point		0.94	0.88	0.87	0.90	0.97

** Significant at the 1 per cent level.

For each method the dry yield per plot was determined and a separate variance analysis was conducted. Table 6 presents the average dry yield for each species, as determined by the various methods, and also includes the F values and the least significant differences as determined by the variance analyses.

The species in Table 6 have been arranged in order of highest yield according to the check method of sampling, the highest yielding species being designated with the letter A. It is seen that the order of the species for Methods 1, 2, and 4 is the same as given by the standard or check method of sampling. In Method 3 a slight change occurred which resulted in species F yielding slightly more than species E. The difference in yield between these two species is not significant. Throughout the whole table there is a great deal of similarity between all sets of yield data. The F values are all at much the same level of significance. Some differences do occur, however, in the significant relationships between species. For example, the check method shows no significant difference existing between the species E and G, but Methods 1, 2, and 3 show that the difference in yield between these two species is highly significant.

In order to further compare the different methods with the check method of sampling, the dry yield data, as determined by each sampling method, were analysed in conjunction with the dry yields of the check method using the split-plot type of variance analysis for each comparison. The summary of these variance analyses is presented in Table 7.

Table 7 shows that Methods 1, 2, and 3 each gives a significantly different average yield than that given by the check method of sampling. It is also seen that when each of these methods was compared to the check

TABLE 7.—SUMMARY OF THE VARIANCE ANALYSES OF THE DRY YIELD DATA, OF THE GRASS SPECIES TEST, AS DETERMINED BY EACH SAMPLING METHOD, IN DIRECT COMPARISON WITH THE DRY YIELD DATA DETERMINED BY THE CHECK METHOD OF SAMPLING

Variation due to	Degrees of freedom	Mean squares — Sampling methods in comparison with check			
		1	2	3	4
Species	6	20.3344**	20.9094**	19.4833**	21.8249**
Replicates	5	6.9456**	6.7487**	6.7463**	6.8100**
Error (*a*)	30	0.7042	0.6669	0.6589	0.6807
Plots	41	—	—	—	—
Methods	1	0.4060**	0.1009*	0.1360**	0.0009
Methods × species	6	0.0947**	0.0462*	0.1702**	0.0280
Error (*b*)	35	0.0183	0.0144	0.0161	0.0177
Total	83	—	—	—	—

* Significant at the 5 per cent point.
** Significant at the 1 per cent point.

method the interaction of methods X species was significant. This indicates that the relationships between the species, as determined by the check method of sampling, were significantly changed when sampling Methods 1, 2 and 3 were used. It can therefore be stated that, for this particular test, sampling Methods 1, 2, and 3 were unreliable. When Method 4 was compared to the check method it was found that no significant difference existed between the two methods nor was there a significant interaction. This is a very good indication that Method 4 was as reliable as the check method of sampling for this particular comparative test.

Sweet Clover Variety Test

The second comparative test used in the sampling study was a sweet clover variety test. The summary of the dry matter percentages, as determined from the different sizes of samples for each variety, appears in Table 8.

In this test the plot of the Improved Alpha variety in the sixth replicate was missing. The method of analysis used was that set forth by Anderson (1) whereby the whole-plot treatments, varieties in this instance, were analysed by estimating the missing whole-plot, and the sub-plot treatments, that of samples, were analysed by the method of proportionate sub-class numbers, in which only the existing plots were considered. Table 8a shows the variance analyses of both the whole-plot and sub-plot treatments.

It is seen that the varieties differed very significantly in the amount of dry matter that they contained, with Erector giving the highest average percentage, 40.9 per cent, and Arctic the lowest average percentage dry matter, 29.0 per cent. Erector was significantly higher than all other varieties and Aura was significantly higher than the remaining varieties. The analysis of variance also showed the replicates to be significantly different.

Table 8.—Dry matter percentages, average of six replicates, as determined by different sizes of moisture samples for six sweet clover varieties

Varieties	Size of sample and sample number							Varietal means
	0.5-pound			0.75-pound		1.5-pound		
	1a	1b	1c	2a	2b	3a	3b	
Erector	40.2	40.5	40.4	41.2	40.5	41.3	42.0	40.9
Aura	35.7	36.6	35.4	36.1	36.0	36.8	37.1	36.2
Redfield Yellow	31.0	31.3	31.8	30.0	30.6	30.6	31.5	31.0
Improved Alpha*	30.6	30.0	30.0	28.8	29.7	29.7	30.2	29.9
M. alba S-567	30.6	28.9	29.3	28.9	30.0	30.4	30.3	29.8
Arctic	29.2	28.2	29.0	29.1	28.6	29.4	29.3	29.0
Sample means	32.9	32.7	32.7	32.5	32.7	33.1	33.5	
Means of sample size	32.8			32.6		33.3		

* Averages of five replicates.
Least significant difference between varietal means at the 1 per cent level = 3.4 per cent.
Least significant difference between means of sample sizes at the 5 per cent level = 0.5 per cent.

TABLE 8a.—VARIANCE ANALYSIS OF THE DRY MATTER PERCENTAGES FROM THE SWEET CLOVER VARIETY TEST

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Whole-plot treatments				
Replicates	5	614.87	122.97	4.08**
Varieties	5	4749.57	949.91	31.51
Error (*a*)	24	723.63	30.15	
Total	34	6088.07		
Sub-plot treatments				
Whole plots	34	6024.73		
Between sample sizes	2	20.70	10.35	4.14*
Within sample sizes	4	4.27	1.07	
Strains × between samples	10	20.80	2.08	
Strains × within samples	20	29.58	1.48	
Error (*b*)	174	434.20	2.50	
Total	244	6534.28		

* Significant at the 5 per cent level.
** Significant at the 1 per cent level.

A significant difference between sample sizes was found to exist. The 1.5-pound samples showed a significantly higher dry matter percentage than both the 0.75 and the 0.5-pound samples. In this test there appears to be no relationship between size of sample and dry matter percentage such as was found in the previous test of grass species. Neither of the interactions was found to be significant. Therefore the sampling of the different varieties was consistent for each size of sample.

Using the procedure previously described the estimated variances of varietal means on a single plot basis and the relative precision factors were determined for each size of sample with r = 6 and k = 1 to 3. These data have been summarized and presented in Table 9.

TABLE 9.—THE ESTIMATED VARIANCES OF VARIETAL MEANS AND RELATIVE PRECISION FACTORS FOR DIFFERENT NUMBERS OF SAMPLES PER PLOT WITH SIX REPLICATES, FOR DIFFERENT SIZES OF MOISTURE SAMPLES

Sample size (pounds)	Mean square		Estimated variance of a plot A	Estimated variance of a varietal mean and the relative precision factor					
	Experimental Error kA + B	Sampling Error B		k = 1		k = 2		k = 3	
				$V\bar{x}$	P.F.*	$V\bar{x}$	P.F.	$V\bar{x}$	P.F.
0.5	20.395	2.755	5.880	1.439	42	1.210	50	1.133	54
0.75	7.456	2.523	2.467	0.832	73	0.621	98	0.551	110
1.5	7.289	1.325	2.982	0.718	85	0.607	100	0.571	106

* P.F. = Precision factor or estimated efficiency in per cent.

The relative precision factors in Table 9 are expressed in terms of $k = 2$, $r = 6$, for 1.5-pound samples, as 100. It is seen that one 1.5-pound sample per plot in this test is a more efficient method of sampling than three 0.5-pound samples per plot, and also more efficient than one 0.75-pound sample per plot. Compared to two 1.5-pound samples per plot one 1.5-pound sample per plot is 85 per cent as efficient. It is therefore seen that the standard method of sampling has been justified in view of the relatively small increase in efficiency gained from double the amount of moisture sampling.

Again, in order to evaluate possible shorter sampling methods, the same methods as described for the grass species test were applied to the sweet clover yield data. The summary of the average yield data for each variety, as determined by each method, and the F values from the individual variance analyses appear in Table 10. The varieties are arranged in order of highest yield as determined by the check method of sampling.

TABLE 10.—SUMMARY OF THE DRY YIELD DATA OF THE SWEET CLOVER VARIETY TEST, AS DETERMINED BY THE DIFFERENT SAMPLING METHODS, WITH THE F VALUES AND LEAST SIGNIFICANT DIFFERENCES FROM THE VARIANCE ANALYSES INCLUDED

Varieties	Key to varieties	Average yield in pounds per plot METHODS				
		1	2	3	4	Check
Redfield Yellow	A	5.61	5.27	5.12	5.31	5.42
Erector	B	4.90	4.99	4.43	5.14	5.02
Arctic	C	4.30	4.38	4.35	4.40	4.33
Aura	D	3.47	3.25	3.43	3.65	3.41
Improved Alpha	E	3.40	3.33	3.11	3.34	3.32
M. alba S-567	F	3.03	3.12	3.01	3.14	3.16
F. value		4.72**	5.06**	4.10**	4.37**	5.22**
L.S.D. (1)‡		1.82	1.62	1.66	1.76	1.67
L.S.D. (2)†		1.93	1.72	1.76	1.86	1.76

** Significant at the 1 per cent level.
† Least significant difference for comparisons of all varieties except Improved Alpha.
‡ Least significant difference for comparison of Improved Alpha with any other variety.

The ranking of the varieties, Table 10, for sampling Methods 1, 3, and 4 are the same as for the check method. Method 2 shows a small change in the order of the varieties D and E. According to the check method variety A is significantly higher than varieties D, E, and F, and variety B is significantly higher than varieties E and F. Method 1 shows the same significant differences except that variety B in this case is not significantly different to variety E. Method 2 shows variety A with the same significance but variety B in this case is significantly different to varieties D, E, and F. By Method 3, variety A is significantly different to varieties D, E, and F but variety B is not significantly different to any other variety. The fourth method of sampling shows varieties A and B significantly different to varieties E and F but not to D.

In order to find if the above changes were significant the dry yield data determined by each sampling method were separately compared to those of the standard method of sampling using the split-plot type of variance analysis. The summary of these variance analyses appears in Table 11.

Table 11 shows that Method 3 is the only method of sampling that differs significantly from the check method. Since the interaction of methods × varieties is not significant when Methods 1, 2, and 4 are each compared to the check method, it can therefore be concluded that the changes in ranking and in the relationships of the varieties, as previously outlined, are not significant. It can thus be stated that for this sweet clover variety test the moisture sampling Methods 1, 2, and 4, were just as reliable as the standard method of sampling.

TABLE 11.—SUMMARY OF THE VARIANCE ANALYSES OF THE DRY YIELD DATA OF THE SWEET CLOVER VARIETY TEST, AS DETERMINED BY EACH SAMPLING METHOD, IN DIRECT COMPARISON WITH THE DRY YIELD DATA DETERMINED BY THE CHECK METHOD OF SAMPLING

Source of variation	Degrees of freedom	Mean squares: Sampling methods in comparison with check			
		1	2	3	4
Whole-plot treatments					
Replicates	5	32.9451	32.3515	30.3459	32.4588
Varieties	5	11.4756	10.6288	9.7268	10.6768
Error (*a*)	24	2.2688	2.0237	2.0883	2.1950
Total	34				
Sub-plot treatments					
Whole-plots	34				
Methods	1	0.0013	0.0148	0.6762**	0.0588
Methods × varieties	6	0.0388	0.0119	0.1302*	0.0699
Error (*b*)	28	0.0972	0.0801	0.0456	0.0812
Total	69				

* Significant at the 5 per cent level.
** Significant at the 1 per cent level.

Brome Grass Strain Test

The dry matter percentage data of the brome grass strain test are summarized in Tables 12 and 12a. It is seen that the differences in dry matter percentages between strains are highly significant even though the maximum difference between any two means is only 3.8 per cent. There is also a significant difference between the percentages of dry matter determined from the different sizes of samples. The necessary difference between size of sample means is 0.6 per cent; thus the 0.5-pound samples gave a significantly higher dry matter percentages than did the 1.5-pound

samples, the difference between them being 0.7 per cent. Although this difference is shown to be significant it can hardly be called an important difference and a rigid distinction between sample size on the basis of this seems hardly justifiable. However, the results found here are in agreement with those of the test of various grass species, that is, there is a definite trend for dry matter percentage to be inversely proportional to the size of the sample used for the determination.

TABLE 12.—DRY MATTER PERCENTAGES, AVERAGE OF FOUR REPLICATES, AS DETERMINED FROM DIFFERENT SIZES OF MOISTURE SAMPLES FOR SIXTEEN STRAINS OF BROME GRASS

Strains	Size of sample and sample number						Means of strains
	0.5 pound			0.75 pound		1.5 pounds	
	1a	1b	1c	2a	2b	3	
S-1264	39.2	40.8	38.9	40.2	39.6	40.5	39.9
S-1224	40.4	39.8	40.6	38.8	38.8	38.7	39.6
S-1258	39.8	39.2	39.9	39.4	38.7	39.3	39.4
S-1229	40.2	39.0	39.8	39.4	39.2	38.3	39.3
S-1262	40.6	38.3	38.7	38.6	38.3	37.6	38.7
S-1260	39.4	38.5	37.9	39.4	38.3	38.0	38.6
S-1256	37.9	38.9	38.7	38.4	38.6	37.8	38.4
S-1265	38.1	37.1	42.4	37.2	37.2	37.2	38.2
S-1263	38.5	38.3	37.5	38.6	37.8	38.1	38.1
S-1261	37.5	38.6	38.3	37.4	37.5	37.0	37.7
S-1259	37.5	38.8	38.1	37.7	36.8	37.0	37.7
S-1227	36.5	35.8	37.3	37.6	38.4	37.6	37.2
S-1257	37.7	38.3	36.1	37.5	36.4	36.4	37.1
S-1255	36.2	36.0	36.9	37.2	36.4	35.8	36.4
Commercial	36.5	37.1	36.9	36.1	35.3	36.0	36.3
Superior	35.8	36.5	35.8	36.4	36.8	35.6	36.1
Sample means	38.2	38.2	38.4	38.1	37.8	37.6	
Means of sample size		38.3		37.9		37.6	

Least significant difference between means of strains, 1 per cent level = 2.3 per cent.
Least significant difference between means of sample size, 1 per cent level = 0.6 per cent.

TABLE 12a.—VARIANCE ANALYSIS OF THE DRY MATTER PERCENTAGES OF THE BROME GRASS STRAIN TEST

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Strains	15	510.19	34.0127	3.82**
Replicates	3	118.16	39.3867	4.42**
Error (*a*)	45	401.01	8.9113	
Plots	63	1029.36		
Between size of samples	2	25.92	12.9600	5.85**
Within size of samples	3	5.00	1.6667	
Between sample × strains	30	70.53	2.3510	1.06
Within sample × strains	45	132.77	2.9504	1.33
Error (*b*)	240	532.13	2.2172	
Total	383	1795.71		

** Significant at the 1 per cent evel.

Precision factors were determined for the 0.5 and 0.75-pound samples and are presented in Table 13. In this test only one 1.5-pound sample could be taken so that it was not possible to estimate the variance of the strain means for different numbers of 1.5-pound samples.

TABLE 13.—THE ESTIMATED VARIANCES OF STRAIN MEANS AND RELATIVE PRECISION FACTORS FOR DIFFERENT NUMBERS OF SAMPLES PER PLOT WITH 4 REPLICATES, FOR TWO SIZES OF MOISTURE SAMPLES

Sample size (pounds)	Mean square		Estimated variance of a plot A	Estimated variance of a strain mean and relative precision factor					
	Experimental Error kA + B	Sampling Error B		k = 1		k = 2		k = 3	
				$V_{\bar{x}}$	P.F.*	$V_{\bar{x}}$	P.F.	$V_{\bar{x}}$	P.F.
0.5	8.33	3.13	1.73	1.22	40	0.82	60	0.69	71
0.75	3.94	1.18	1.38	0.64	77	0.49	100	0.44	111

* P.F. = Precision factor or efficiency in per cent.

The relative precision factors in Table 13 are expressed in terms of k = 2, r = 4, for 0.75-pound samples, as 100. It is seen that one 0.75-pound sample per plot is more efficient than three 0.5-pound samples per plot, thus further indicating that the smaller sizes of moisture samples are more variable and hence less reliable than the larger moisture samples.

The shorter methods of sampling as used in the two previous tests were also applied to the yield data from the brome grass strain test. In this test, however, there were only four replicates; thus some changes in the sampling methods were necessary. In Method 1 the random 0.5-pound samples from each plot were averaged for the first two replicates for each strain and similarly for the second two replicates. Thus this method in actual use would mean drying two 1-pound samples for each strain instead of two 1.5-pound samples. Methods 2, 3, and 4 were the same as previously described. A further method, number 5, was added to this test. This consisted of taking six random 1.5-pound samples for the whole test, averaging them, and determining the dry yield of each plot on the basis of the average dry matter percentage. The results of the variance analysis of the yield data on the basis of this method of sampling would be essentially the same as they would be if green yields had been used.

The summary of the yield data, as determined by the different methods of sampling, and the F values from the variance analyses of the dry yield data for each method, are presented in Table 14. The strains are listed in order of highest yield as determined by the check method of sampling.

It is seen that there are no significant differences between strains for check method or for Methods 2 and 3, although in all cases the 5 per cent level of significance is approached. Sampling methods 1, 4 and 5 all show the strains to be significant to the 5 per cent point. Several changes occur in the ranking of the strains for yield.

The dry yield data from each sampling method were compared to the dry yields from the standard method of sampling using the split-plot type of variance analysis. The summary of these analyses is presented in Table 15.

Table 14.—Summary of the dry yield data of the brome grass strain test, as determined by the different sampling methods, with the F values and least significant differences from the variance analyses included

Strains	Methods of sampling					
	1	2	3	4	5	Check
S-1265	3.60	3.61	3.22	3.85	3.71	3.66
S-1263	3.56	3.62	3.69	3.55	3.55	3.60
S-1264	3.29	3.24	3.26	3.56	3.19	3.43
S-1258	3.39	3.36	3.19	3.40	3.24	3.38
Superior	3.30	3.43	3.26	3.41	3.54	3.32
Commercial	3.38	3.29	3.50	3.22	3.42	3.27
S-1256	3.26	3.30	3.15	3.22	3.21	3.23
S-1255	3.30	3.30	3.26	3.27	3.40	3.23
S-1260	3.06	3.06	3.20	3.06	3.04	3.07
S-1259	3.15	3.05	2.98	3.15	3.11	3.05
S-1229	3.05	3.14	3.02	3.11	3.02	3.05
S-1257	3.03	3.04	2.97	3.03	3.06	2.95
S-1261	2.96	2.94	2.81	2.97	2.93	2.87
S-1224	2.63	2.56	2.50	2.60	2.50	2.55
S-1262	2.42	2.40	2.38	2.37	2.38	2.36
S-1227	2.19	2.30	2.26	2.27	2.25	2.25
F value	1.98*	1.76	1.88	2.14*	2.13*	1.68
L.S.D.	0.80	—	—	0.83	0.82	—

* Significant at the 5 per cent level.

Table 15 shows that by using sampling methods 3 and 4 a significantly different average yield is obtained. A slightly different average yield is not a very important factor, particularly when the difference is small. Methods 3 and 5 bring about a significant interaction between methods × strains. This indicates that the yield relationships between the various strains are changed significantly from that of the check method. Therefore

Table 15.—Summary of the variance analyses of the dry yield data, of the brome grass strain test, as determined by each sampling method, in direct comparison with the dry yield data determined by the check method of sampling

Variation due to	Degrees of freedom	Mean squares — Sampling methods in comparison with check				
		1	2	3	4	5
Strains	15	1.2787	1.2719	1.2322	1.3834	1.3490
Replicates	3	3.9695	3.9225**	4.0462**	4.1454**	4.1076**
Error (*a*)	45	0.7085	0.7440	0.7202	0.7340	0.7270
Plots	63					
Methods	1	0.0118	0.0218	0.0473*	0.0775**	0.0093
Methods × strains	15	0.0097	0.0109	0.0456**	0.0087	0.0264**
Error (*b*)	48	0.0087	0.0076	0.0095	0.0095	0.0099
Total	127					

* Significant at the 5 per cent point.
** Significant at the 1 per cent point.

Methods 3 and 5 are not sufficiently reliable for this test. Methods 1, 2, and 4 did not produce a significant interaction; therefore any differences found in the relationships between strains when comparing these sampling methods to the check method could be accounted for on the basis of chance variations. It can therefore be stated that for this test the shorter sampling methods 1, 2, and 4, have been as reliable as the standard method of sampling.

DISCUSSION

No data regarding the correlation of dry weights with green weights have been included in this study. Since the green yield of any plot consists of the dry yield plus a variable amount of water, and since the dry yield depends on the green yield and the weight of the water, a correlation between any two of these factors would not be justified. With such a relationship between the variables the correlation coefficient would necessarily be high. If there was a perfect correlation between green and dry yields it could be concluded that green yields would be as reliable as dry weights. However, once the correlation coefficient was less than 1 the reliability of the green yields would be doubtful. This can be illustrated by the following example. Test number 10, Table 5, was a sweet clover variety test, the green yields of which were shown as giving quite different results to that of the dry yields. When the green yields in this test were correlated with the dry yields the coefficient cf correlation was found to be +0.98.

The short sampling methods that were suggested in this paper should be considered from the practical viewpoint. Methods 1 and 2, involving the combination of small amounts of green material, might, in actual practice, be found to be somewhat complicated and subject to considerable error. One such possible error might be the combination of samples from plots of different species or varieties being sampled. It has also been shown that considerable variation in percentage dry matter occurred when the smaller samples were used. This variation might be due, in part at least, to inaccurate field scales. Therefore these methods, although less time-consuming, might prove to be insufficiently reliable.

Sampling methods 3 and 4 would be very desirable methods since they are relatively easy to employ and would considerably reduce the number of moisture samples. It has been shown, however, that Method 3 in no case gave results that were as reliable as the standard method of sampling. Method 4 on the other hand, in all three tests studied, consistently gave results that were as reliable as the standard method. It thus appears that this latter method of sampling, which consists of sampling two random replicates, using the standard method, and averaging the two dry matter determinations for each variety or treatment, has definite possibilities. It would be highly desirable, however, to test this method again in other years, under different growing conditions, and using different comparative tests.

In dealing with the one-crop tests earlier in the study the conclusion was drawn to the effect that reliable comparisons in the one-crop tests could be made on the basis of green weights, or, for year comparisons, on dry weights based on the average of a few random 1.5-pound moisture

samples. Since the brome grass strain test was a one-crop test it seemed desirable to test that suggested method of sampling. Table 15 shows that Method 5 was not as reliable as the check method of sampling; thus these results tend to contradict the earlier conclusion. However, when it is considered that the test of brome grass strains is an advanced test of many similar strains of one grass species and that very accurate comparisons are necessary in order to bring out the differences, then one should not expect a non-detailed method of sampling to bring out small differences. The one-crop tests discussed earlier were tests of a preliminary nature where fairly large differences between strains could be expected to be found. In such cases this Method 5 should prove to be as satisfactory as more detailed sampling methods.

SUMMARY

1. Significant differences in dry matter percentages were found to exist between strains, varieties, and species of all of the forage crops studied.

2. Significant differences between strains or varieties of a crop did not necessarily justify complete moisture sampling in a comparative test.

3. It was shown that where comparative tests of forage crops for hay yield did not include markedly different varieties or species, then moisture sampling on the basis of one moisture sample per plot was not necessary. Several random samples for the whole test would be sufficient to reduce green yields to dry or hay yields for inter-year comparisons.

4. Where the comparative tests of forage crops for hay yields included more than one species, and where species or varieties differed markedly in dry matter percentage, such as in the case of sweet clover varieties, or where there was an advanced test of many very similar strains, then it was necessary to make moisture determinations on a fairly complete basis.

5. Moisture samples of 0.5 and 0.75 pounds in green weight were found to give significantly different dry matter percentages than the 1.5-pound samples.

6. The smaller moisture samples were shown to be more variable than the larger samples. In one case one 1.5-pound sample per plot was as efficient as three 0.5-pound samples per plot.

7. More than one 1.5-pound sample per plot was found to be unjustified, in the material tested.

8. Moisture sampling on the basis of one 1.5-pound sample per plot in two random replicates and averaging of the two determinations for each variety or treatment was found, in the three tests studied, to be essentially as reliable as sampling every plot in the tests.

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VARIATIONS IN THE ESTRONE CONTENT OF PREGNANT MARES' URINE[1]

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INTRODUCTION

The examination of mares' urine samples taken between the fifth and ninth months of pregnancy disclosed wide variations in the concentration of estrone for mares at the same stage of pregnancy. It was decided to determine the urinary estrone concentration for a single mare throughout the entire gestation period and to compare these values with those for the previously-examined mares at identical stages of pregnancy.

Limited literature references to the estrone content of pregnant mares' urine (P.M.U.) disclose wide variations and infer that the values obtained depend on the method applied. Cole and Hart (3), employing a rat test, report a maximum estrone value of 17,500 rat units (17.5 mg.) per litre of urine at 240 days of pregnancy. Cole and Saunders (2) report maximum concentrations of 16,000 and 33,000 rat units at 200 to 275 days. Mayer *et al.* (5) using a chemical technique, in contrast to the above bioassay techniques, found 17.1 mg. of estrone per litre at the fortieth day of pregnancy. Selye (6) reports a concentration of 100 milligrams per litre but the method applied is not stated. The data to be reported are based on a chemical method involving colorimetry.

PROCEDURE

Collection of Urine

Since the mare under study was a working horse, only overnight collections were made. The collection equipment consisted essentially of a heavy curved rubber tube held in position by a light canvas harness. Specimens were obtained at approximately weekly intervals and averaged about one-half gallon in volume. When specimens were lost during the collection period or were low in volume another specimen was secured on the following night. As the collecting equipment did not always permit total retention of urine, no measurements of urine volume were recorded.

Determination of Estrone

The method used for the determination of estrone is based on modifications of the methods of Kober (4), Venning *et al.* (7) and Bachman and Pettit (1). An acid hydrolysis of the urine to obtain the hormone in the free form is followed by several benzol extractions. The combined benzol extracts are washed successively with water, bicarbonate, carbonate, sulphuric acid, and water and then made up to volume. Suitable aliquots, depending on the hormone concentration, are allowed to react with phenol-sulphonic acid reagent in the presence of heat. After cooling, the addition of water, and successive boiling and cooling, a definite amount of sulphuric acid is added to develop the characteristic pink colour. Readings taken

[1] Contribution No. 165 from the Division of Chemistry, Science Service, Department of Agriculture, Ottawa, Canada.

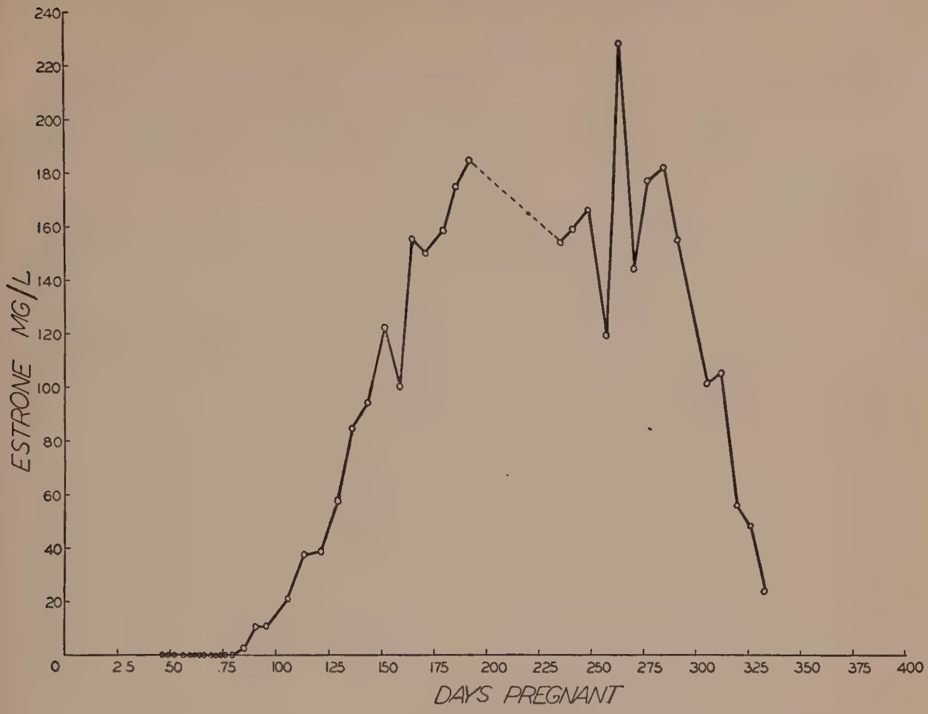

FIGURE 1. The urinary estrone concentration of Mare 10 throughout pregnancy.

NOTE.—*Due to technical difficulties data were not obtainable between the 192nd and 235th days.*

on the Evelyn colorimeter at 515 and 420mμ permit calculation of estrone values corrected for the brown coloration caused by impurities. For ease in calculation, estrone values of urine samples were determined directly from a nomogram based on international standard estone.

RESULTS

The data obtained from urine specimens for a single mare (Mare 10) for the entire gestation period are shown graphically in Figure 1 expressed as milligrams of estrone per litre.

Frequent specimens were examined early in pregnancy but the first detectable amount of the hormone was noted on the eighty-fifth day. From this point the analyses of weekly specimens revealed a rapid increase in estrone concentration until about the 190th day of pregnancy. During the next hundred days the estrone level, while subject to considerable variation, was maintained at an average value of 170 mg./l. A marked decrease in production occurred during the last forty-eight days of gestation and the low value of 24 mg./l was recorded on the day of parturition.

These variations in estrone concentration for Mare 10 follow the same pattern as that found by Cole and Hart (3) but the values obtained are considerably higher than those of the above workers for the entire gestation period. Mayer *et al.* (5) report an earlier appearance of estrone than was found for Mare 10 and a larger amount at the fortieth day of pregnancy.

The estrone values for Mare 10 are compared in Table 1 with data from other P.M.U. specimens obtained from mares at known stages of pregnancy. In making the comparison it is assumed that no marked change occurs in the hormone production rate over a twenty-four hour period and therefore the time of collection of a specimen would have no appreciable effect on its concentration.

Foaling data for these mares indicate that no abnormal gestations occurred.

TABLE 1.—INDIVIDUAL VARIATIONS IN EQUINE URINARY ESTRONE

Mare No.	Days pregnant	Estrone, mg./l	Estrone mare 10*, mg./l
106	157	89	105
21	158	142	101
78	161	102	119
77	162	63	141
115	164	220	156
19	176	160	158
69	199	114	178
149	202	103	175
35	229	100	155
109	242	51	160
137	271	33	145

* Corresponding days pregnant.

It is evident that great variations exist in the amounts of estrone excreted by different mares at the same stage of pregnancy. For example, the estrone concentrations for the first five mares of the above table vary from 63 to 220 mg./l although the urine specimens were collected within the same week of pregnancy.

While the above data refer to randomly selected specimens it is evident that the maximum production of estrone does not always occur at the same time in different mares. This observation is in agreement with the findings of Cole and Saunders (2) who report maximum concentrations at 158, 207, 220 and 270 days.

SUMMARY

The urinary estrone excretion throughout the entire gestation period of a single mare has been determined by a chemical method. Comparisons have been made between the estrone values for this mare and for other mares at the same stage of pregnancy.

The concentration of urinary estrone for a single pregnant mare rose rapidly from the low value of 2.2 mg./l at the 85th day to an average maximum value of 170 mg./l between the 192nd and 235th days. Thereafter it declined steadily to a value of 24 mg./l on the day of parturition.

Great variations exist in the concentration of estrone excreted by different mares at identical stages of pregnancy.

The maximum production of estrone apparently does not always occur at the same time in different mares.

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CHLOROTIC BANDING OF CEREAL SEEDLINGS[1]

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INTRODUCTION

Chlorotic banding of the leaves of cereal seedlings (Figure 1) is of common occurrence in the spring-wheat region of the Canadian Prairies (3). Hitherto, chlorosis of the banded type has been generally attributed to temperatures at or near the freezing point, frequently with strong winds as an associated factor. Machacek (5), however, reports a case of yellow banding of wheat leaves caused by extreme heat at the soil level. A more severe type of injury in which the tissues are killed, with death of the seedlings often resulting, is frequently encountered. This is usually caused by severe frosts (2). A similar type of injury on late-sown spring oats was attributed by Vanterpool (4) to unseasonable, hot, dry winds one or two days after the plants emerged.

Beginning about May 24, 1948, and for several days thereafter, reports were received of conspicuous yellowing and banding of wheat seedlings over large areas in a general region around Saskatoon and extending northwards and eastwards for about 100 miles. Spring sowing was late. Because of the lack of rain, the surface soil had dried out to seed depth or lower in many fields by the latter part of May. The result was successive periods of seedling emergence in the same field. Unseasonably high air temperatures (Table 2) from May 17 to May 25, and the dry surface soil, strongly indicated that heat rather than cold was the primary contributing factor in last year's yellow banding of wheat seedlings.

Because of the wide distribution of this trouble in north-central Saskatchewan, and the scarcity of published information on this type of heat injury on cereal seedlings, some exploratory work was conducted on the problem in the summer of 1948. The results of the experiments are presented here.

TYPES OF INJURY

Richards (10) has given detailed descriptions of three types of lesions on young cereal plants produced by temperatures at or near the freezing point at the soil surface. The first consists of yellow, chlorotic bands on the first and second leaves, varying from a small speck to bands up to three-fourths of an inch in length (cf. Figure 1). In the second type of injury the banded tissue is bleached and dead, commonly resulting in a characteristic breaking or lopping over of the leaves by subsequent strong winds. The third type of injury consists of a complete collapse of tissue at the soil line. This is the most damaging kind. It is sometimes called the "damping-off" type. Richards has pointed out that the various types are caused by different degrees of intensity of cold at a common point of origin, that is, the soil surface. This is borne out by the author's observations.

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[1] Contribution from the Laboratory of Plant Pathology, University of Saskatchewan, Saskatoon, Sask., with financial assistance from the Saskatchewan Agricultural Research Foundation.

[2] Professor of Plant Pathology.

TABLE 1.—MINIMUM AIR TEMPERATURES, SASKATOON, MAY, 1930.

Date	Minimum temperature	Date	Minimum temperature
May	°C.	May	°C.
12	5.3	16	−5.5
13	0.3	17	−0.1
14	−1.3	18	5.3
15	0.5		

TABLE 2.—DAILY RECORD OF METEOROLOGICAL DATA AT SASKATOON, MAY 17 TO 25, 1948.

Date	Maximum air temperatures	Sunshine	Evaporation	Wind
May	°C.	hr.	in.	M.P.H.
17	25.8	9.3	0.096	23.5
18	27.9	11.4	0.160	13.8
19	23.6	9.3	0.192	16.8
20	24.9	14.7	0.096	2.1
21	28.5	14.3	0.192	7.1
22	28.0	14.5	0.192	18.4
23	24.4	14.1	0.160	15.5
24	26.0	15.5	0.384	4.3
25	29.0	14.3	0.220	5.5

FROST BANDING OF SEEDLINGS IN 1930

Chlorotic banding of wheat and barley (Figure 1) seedlings with symptoms as described above were first observed by the author in May, 1930, at Saskatoon, following a period of successive nights when the minimum air temperatures, at the 4-ft. level, were around the freezing point (Table 1). Since the average minimum "grass" temperature for May, at Saskatoon, is about 1.6° C. lower than the corresponding air temperature, it is highly probable that the temperatures at the surface of the soil fell below the freezing-point on May 13 and 15. The effects of these four or five successive freezing temperatures at soil level on barley seedlings photographed on May 18 are shown in Figure 1. The banding was usually more conspicuous on barley than on wheat seedlings. A gradual greening of the chlorotic bands was observed to occur.

HEAT BANDING OF SEEDLINGS IN 1948

The wide distribution of chlorotic banding and general yellowing of cereal seedlings over considerable areas of north-central Saskatchewan has already been mentioned. A slight retardation in growth was suspected in fields showing the more severe types of injury.

On May 26, two samples of wheat seedlings with clumps of adhering soil were received from the Soils Department, University of Saskatchewan. One sample contained many seedlings which showed the chlorotic type of

banding with an occasional band of the bleached, dead type. This was collected from a loose, dry area of a silty, clay loam field. In the other sample the seedlings appeared normal and were collected from the same field where the soil was moist. The samples were collected on May 25. Meteorological data for May 17 to 25, 1948, are given in Table 2.

Both banded and normal seedlings were planted in separate flat boxes in the greenhouse and kept moist. The chlorotic bands of living tissue gradually turned a normal green colour, so that after five or six days in most instances it was difficult to detect the former position of the chlorotic bands. The bleached, white bands, however, failed to recover and leaves with this type remained broken over. In the field the tops of such plants would blow as flags in the wind.

The foregoing observations indicated that heat and not cold was responsible for the chlorotic banding of cereal seedlings in 1948, that dry surface favoured the banding, and that subsequent greening of the chlorotic bands occurred, but that the bleached bands failed to recover.

EXPERIMENTS ON HEAT BANDING OF CEREAL SEEDLINGS

Experimental Series I

The following preliminary experiments were conducted in early July to ascertain whether or not high temperatures were responsible for the banding.

Thatcher wheat was sown in boxes on the surface of moist soil, half of which were then covered with $1\frac{1}{2}$ in. of air-dried soil and half with moist soil. Half of each series was placed outdoors and half in a greenhouse with the glass roof lightly coated with a white wash.

Chlorotic banding developed only on the leaves of some seedlings in the outdoor boxes with the dry surface soil from one to four days after emergence. Those in the boxes with moist surface soil were normal green. Greening of the yellow banded seedlings occurred in the course of a few days. During the first few days after the seedlings emerged, the outside maximum air temperatures varied between 26° and 28° C., while in the greenhouse they were 5° to 8° C. higher. Afternoon surface-soil temperatures were around 45° C. in the boxes with dry surface soil in the open, and 4° to 11° C. lower in the boxes with moist surface soil.

These results indicated that direct insolation on dry surface soil was necessary for the production of heat banding on wheat and that high air temperatures alone, as occurred in the greenhouse, were not responsible. The cooling effect of evaporation from the surface of the moist soil kept the temperature below the critical point for chlorotic banding.

Experimental Series II

Further experiments were then conducted in the open to ascertain the surface-soil temperatures at which heat banding occurred. Wooden boxes, 12 in. × 12 in. × 10 in., were filled to within $1\frac{1}{2}$ in. of the top with a moist clay-loam field soil. Thatcher wheat was sown half an inch apart in rows 3 in. apart. The boxes were then filled to the top with air-dried soil, and were buried in the open so that their tops were level with the surface of the soil. Insolation and wind effect were much the same as in an open field.

In order that radiation effects would not be interfered with, all boxes were kept uncovered and the experiment repeated at intervals of a few days so that the chances of continuous fine weather with reasonably high maximum daily temperatures during the first few days after emergence would be met. Surface-soil temperatures were taken with ordinary glass centigrade thermometers placed at an angle of from 5° to 10° to the soil surface so that the mercury bulb was covered with a layer of soil from 2 to 3 mm. thick. There were two thermometers to each box. Temperature readings were recorded hourly from 12.30 p.m. to 3.30 p.m. from the day after the seedlings emerged until they were about four inches high. The highest average reading of the two thermometers of a box at a given time was taken as the maximum temperature for that box. The temperature variations in a given box were surprisingly low, seldom exceeding 5° C. It was felt that the method used for measuring the soil temperature was sufficiently accurate for the purposes of the study.

An experimental series, consisting of from two to four boxes, was set up on eight different dates during July and August. Four experiments, one of which included oats and barley, were successfully completed during periods of clear, warm weather; the remainder were spoiled by rain or cool, cloudy weather.

Daily records of maximum surface-soil temperatures in the experimental boxes and some meteorological data beginning with the date of seedling emergence for three experiments are given in Table 3. It will be seen that surface-soil temperatures of from 45° to 52° C. were recorded within an air temperature range of from 24° to 35° C. Figure 2 shows wheat seedlings from Series II, Experiment 1, with typical chlorotic heat-banding effects and the corresponding dates on which they were initiated. In this experiment, emergence began on July 31; the seedlings were removed for photographing at 11.45 a.m. on August 4. Chlorotic banding was the main type of injury in this experiment, with traces of whitespot burning at the tips and on one side of some leaves.

TABLE 3.—DAILY RECORD OF MAXIMUM SURFACE-SOIL TEMPERATURE AND OF METEOROLOGICAL DATA AT SASKATOON, 1948.

Date	Maximum surface soil temperatures	Maximum air temperatures	Sunshine	Evaporation	Wind
SERIES II.	°C.	°C.	hr.	in.	M.P.H.
Experiment 1.					
July 31	41 – 45	23.3	14.2	0.160	10.6
Aug. 1	44 – 52	24.8	10.2	0.128	3.8
Aug. 2	45 – 52	24.3	14.6	0.096	8.5
Aug. 3	46 – 52	26.3	11.4	0.160	12.6
Experiment 2.					
Aug. 7	42 – 46	26.6	10.1	0.160	7.1
Aug. 8	39 – 43	26.6	11.4	0.124	9.1
Experiment 3.					
Aug. 17	45 – 50	28.3	14.1	0.096	5.5
Aug. 18	45 – 52	34.8	11.6	0.224	11.6

In Series II, Experiment 2, leaf damage was again predominantly of the chlorotic banded type with, in addition, traces of leaf-tip burning on August 7, and a single case of burning on one side of an otherwise chlorotic lesion on August 8.

Series II, Experiment 3, contained Montcalm barley and Victory oats as well as Thatcher wheat. Leaf-tip chlorosis was produced in all the cereals on August 17, when emergence was general; in addition, there was some tip burning in barley. On August 18, chlorotic banding was abundant and burned lesions moderate.

The results of the various series show that cereal seedlings from one to four days after emergence are susceptible to chlorotic banding when the surface-soil temperatures are at least from 42° to 45° C., while from 45° to 52° C. bleaching or burning of the tissues becomes more common, and collapse and death of a few seedlings may occur at from 52° to 54° C. No injury was observed on seedlings where the surface-soil temperatures were below 42° C. Greening or recovery of the chlorotic bands takes from three to ten days or more. Montcalm barley was more sensitive to heat injury than Thatcher wheat or Victory oats. It is interesting to note that Olson (8) found Montcalm barley to be more susceptible than Mindum and Renown wheats to spring-frost injury and slightly more so than Vanguard oats.

The types of heat injury produced on cereal seedlings between surface-soil temperature limits of 42° C. and 52° C. are virtually indistinguishable from those produced by temperatures at or near the freezing point (cf. Figures 1 and 2).

DISCUSSION

It is interesting, physiologically, that temperature extremes at the soil surface can bring about the same abnormal effects or symptoms on cereal seedlings. This is probably due to the effects of temperature on the mechanism of chlorophyll formation. Thus Lubimenko and Hubbenet (7) have shown that the greening process of etiolated wheat seedlings takes place between temperature limits of about 2° C. and 48° C. These points do not depend upon the seedlings' exposure to light. In the view of these workers, temperature is related to the greening process through its influence on the synthesis of leucophyll and its transformation into chlorophyllogen, both of which processes are dark reactions. The transformation of chlorophyllogen into chlorophyll is a photochemical reaction. In Eyster's (6) view, there is only one precursor of chlorophyll, protochlorophyll, which through photooxidation is transformed into chlorophyll. It appears that in chlorotic banding of the leaves of cereal seedlings, whether caused by low or by high temperatures, the mechanism, probably enzymatic, controlling the formation of one or more precursors of chlorophyll has been interfered with. This mechanism may take a few to ten days or more for complete recovery. Desiccation of the tissues may be one factor involved, as this is known to inhibit chlorophyll synthesis. Regardless of the number of precursors, it seems that the temperature extremes have strongly influenced the mechanism for the development of chlorophyll precursors, especially in delaying recovery.

As far as is known, the close similarity of the three symptom types produced on cereal seedlings by surface-soil temperatures just above the

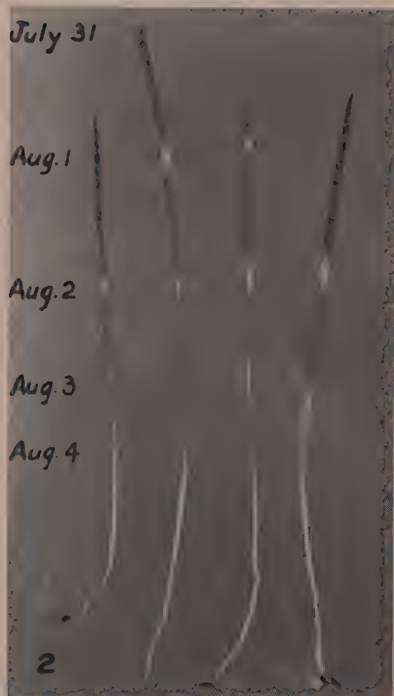

FIGURE 1. Chlorotic banding of fresh barley seedlings caused by slight frost at the soil surface on consecutive nights, May 13 to 17, 1930.

FIGURE 2. Chlorotic banding of dried wheat seedlings caused by heat (42° to 52°C.) at the soil surface on consecutive afternoons, July 31 to August 4, 1943.

minimum and below the maximum for their growth has not previously been pointed out. Attention is drawn to an analogous relationship between the similarity of some forms of "whitetip" or "ear tipping" damage of wheat spikes caused by frost and that caused by heat and drought. This similarity has previously been pointed out by Bell (1) and others.

Reddy and Brentzel (9) consider the critical temperature for heat canker of flax to be at about 54° C. They quote Mayr, and Münch, for the fact that the death point for vegetative cells lies at about 54° C. It seems probable, therefore, that the actual temperatures in the tissues of the seedlings in the experiments reported here may be slightly higher than the surface-soil temperatures recorded by the mercury thermometers.

Unless soil moisture is favourable, late-sown spring cereal seedlings on the Prairies are liable to injury from high surface-soil temperatures. A slight increase in the rate of sowing for late-sown cereals should be a good precautionary measure.

SUMMARY

Chlorotic banding, whitespot banding, and injury similar to damping-off were produced on spring-sown cereal seedlings by high surface-soil temperatures one to four days after emergence. Chlorosis first appeared when these temperatures registered from 42° to 45° C. (108° to 113° F.); whitespot injury increased as the temperature rose to 52° C. (125° F.); and collapse and death occurred at about from 52° to 54° C. (125° to 129° F.) and higher. These soil temperatures prevailed on clear summer days when air temperatures ranged from 24° to 35° C. (75° to 95° F.). Greening or recovery of the chlorotic bands took a few to ten days or more. The whitespot or bleached lesions did not recover, but later development of affected seedlings was normal. Injury to the growing points resulting in collapse was fatal. The same three types of symptoms are commonly found on seedlings which have been subjected to surface-soil temperatures at or near the freezing-point. Thus the same physiological effects on the development of chlorophyll and its precursors in spring-sown cereal seedlings can be produced by temperatures at or near the minimal and maximal cardinal points for growth. Slightly higher rates of sowing for late-sown cereals should insure against reduction in stand by heat.

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SULFATHIAZOLE IN RELATION TO AMERICAN FOULBROOD[1]

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The use of sulfathiazole in the treatment of American foulbrood and the controversies over "pros" and "cons" have received much publicity in the trade journals. Comparatively little has appeared on this subject in the scientific literature based on carefully controlled experiments. What has appeared, however, has demonstrated beyond question the prophylactic and therapeutic effect of sulfa drugs on American foulbrood. At the same time, practically every scientific publication reports recurrences of infection, in some cases as high as 30 to 50 per cent, when treatments with sulfa syrup are discontinued. In consequence, a host of problems have presented themselves—the resistance of American foulbrood spores to these drugs, the influence of these drugs on the vegetative cells of *Bacillus larvae*, the etiological agent, the possible adaptation of the cells to the drugs; and in the apiary, continuous versus restricted feeding of medicated syrup, spraying of the drugs, adulteration of surplus honey, installation of package bees, masking and spread of the disease, period of isolation of infected, treated colonies, etc. As the following brief review of the literature will show, some of these problems have been resolved, others are being or remain to be studied.

Haseman and Childers (7) first reported that the feeding of sulfa drugs in sugar syrup or pollen substitute to bees could prevent the development of American foulbrood and that it resulted in the cleaning up of infected colonies. They claimed too that sulfathiazole "has a very beneficial action on bees infected with the Nosema parasite"—an observation which has not as yet been verified. Certain badly infected American foulbrood colonies were not cleaned up completely and when feeding was discontinued for two months, the disease reappeared in the late brood; these colonies finally died. In a later paper Haseman (5) reported that sulfaguanidine is as effective as sulfathiazole (and sulfanilamide). Furthermore, he stated that a thorough clean-up with sulfa may protect a colony against re-infection for two seasons but advised feeding the drug each spring and fall, or whenever infection appeared, as part of a regular apiary routine. In his most recent publication (6) he stated that streptomycin and penicillin may be used successfully but involve too much trouble.

Milne (13, 14) obtained essentially similar results but noted recurrence within 6 weeks of cessation of feeding. More important still, he reported that some colonies did not react successfully to treatment. Out of 32 colonies treated, however, 29 showed no evidence of disease during the period of treatment (33 to 148 days). Johnson and Stadel (11) and Johnson (9, 10) reported favorable results with 6 out of 7 colonies (among others) variously infected; three showed recurrence in the following season. Five out of 6 hives remained free of disease for two full years after the original treatment

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with sulfa, and 10 out of 11 passed through one year without a definite recurrence. Pen cillin, furacin and x-ray treatment were ineffective; sulfaguanidine was much slower than sulfathiazole, but sulfadiazine and sodium sulfathiazole were as effective as sulfathiazole. Eckert (2, 3), using sodium sulfathiazole, observed recurrence with 2 out of 19 colonies, although further treatment resulted in the elimination of all visual signs of disease from these two colonies; the remaining colonies reacted favorably, some within a short time, wintered well and were clean the following season. Eighteen other infected colonies were successfully treated with sulfa and remained apparently clean throughout the season. He also reported that European foulbrood, sacbrood or paralysis of bees were not controllable with sulfa drugs.

In laboratory studies on the influence of sulfa drugs on *B. larvae*, Katznelson (12) showed that different strains responded differently to various concentrations of sodium sulfathiazole but that most were killed in a 1 per cent solution. This concentration did not affect the spores when suspended in water or 75 per cent honey for over 16 months. The organisms could be adapted to grow in 1 per cent of the drug but lost their resistance rapidly on being subcultured in absence of the drug. Certain antibiotic agents such as penicillin and notatin inhibited *B. larvae* strains in dilutions up to 1:5 or 1:10 million. The results reported herein represent experiments on the effect of sodium sulfathiazole on American foulbrood in the apiary.

EXPERIMENTAL

In the spring of 1948 experiments were set up to study both the prevention of infection and the cleaning up of infected colonies by sodium sulfathiazole. The drug was fed in the usual manner (from an inverted, perforated honey pail placed directly over the brood combs) at the rate of 0.5 gm. per gallon of a 1:1 sugar-water syrup; one gallon only was used per colony. American foulbrood spores were fed in the same syrup at the rate of about 2 billion per gallon. Each treatment was carried out in duplicate. In addition, p-aminobenzoic acid, the most potent sulfa-drug antagonist known (8) was fed to other colonies with and without sulfa at the rate of 0.5 gm. per gallon. At the same time, another experiment was carried out

TABLE 1.—INFLUENCE OF SODIUM SULFATHIAZOLE ON INFECTION OF BEE LARVAE WITH AFB SPORES

Treatment of 50 per cent sugar syrup April 4, 1948	Results
1. AFB spores	Heavy infection
2. " " + PABA*	Heavy infection
3. " " + sodium sulfathiazole	No infection
4. " " + PABA + sodium sulfathiazole	Infection
5. AFB spores kept in 1 per cent sodium sulfathiazole solution in water or honey for 4 months	Infection
6. No treatment	No infection
Infected colonies from treatments 1, 2, 4, 5, fed drug June 1-15—last examination Oct. 6	No infection

* p-aminobenzoic acid

TABLE 2.—RESULTS OF EXPERIMENTS ON SULFA FEEDING WITH SIX COLONIES OVER A PERIOD OF THREE YEARS*

Colony	1946			1947			1948
	June treated	August examined	Fall treated	June examined	June treated	Fall examined	June and fall examined
117	Sulfa	–	Sulfa	–	0	–	–
73	"	–	0	–	0	–	–
125	"	–	0	–	0	–	–
17	"	–	0	+	Sulfa	–	–
97	"	+	Sulfa	+	"	–	–
53	"	+	0	+	"	–	–

* Sulfathiazole or sodium sulfathiazole used.
0 = No treatment
+ = Infection
– = No infection

to determine the influence of the drug on the virulence rather than the viability of American foulbrood spores. Burnside (1) has reported that American foulbrood spores may be heated to a point where their virulence is destroyed but not their ability to germinate and produce growth in a suitable medium. Spores were kept in a 1 per cent solution of sodium sulfathiazole in both water and honey; they were then washed several times to remove the sulfa and fed in 50 per cent syrup. The results given in Table 1 show clearly that the drug prevented infection of the larvae and that p-aminobenzoic acid neutralized its action, thereby permitting infection to develop. P-aminobenzoic acid itself had no effect on American foulbrood spores nor did their immersion in 1 per cent sodium sulfathiazole solution for four months prior to testing.

All colonies showing infection were then given 1 gallon of medicated syrup and within 6-8 weeks they showed no sign of disease, remaining clean up to the last examination on October 6, 1948. Naturally it required a longer period for the badly infected colonies to be cleaned up. The infected material removed by the bees is dropped largely in front of the hive and constitutes a source of future infection of the colony although the material may be dispersed by rain or melting snow.

Another series of experiments had been set up in 1946*. Ten colonies were infected with American foulbrood combs after which two received sulfathiazole in tablet form, two sulfathiazole in powder form and two sodium sulfathiazole; three received penicillin and one was left as control. Penicillin failed to check the disease which developed rapidly; these colonies were then fed sulfa syrup and the disease began to disappear and healthy brood to become established. The results with the sulfa drug and subsequent manipulations of the colonies are given in Table 2. Four of the six colonies were cleaned up but two contained a few scales at the end of the season. Sulfa was fed as indicated but in June, 1947, 3 of the 6 colonies, or 50 per cent showed evidence of disease. However, when sulfa was again fed to the

* By Mr. Stephen formerly of the Bee Division, now Extension Beekeeper, State College, Raleigh, North Carolina.

infected colonies, all evidence of the disease disappeared and all 6 colonies were apparently free from it throughout the 1948 season. These colonies are to be kept under observation.

Sulfathiazole was also tested in co-operation with a commercial beekeeper who in June, 1946, placed a total of 27 infected colonies at the disposal of the Bee Division. Medicated syrup was fed throughout the season and on September 25 only two colonies showed signs of the disease. Treated syrup was fed for the winter and again in the spring of 1947 and the colonies remained free from all signs of American foulbrood during this and the 1948 season.

DISCUSSION AND CONCLUSIONS

The results presented above indicate that American foulbrood may be controlled with sulfathiazole when used carefully by experienced workers. Whether the rank-and-file beekeeper can do this remains problematical. The human element was perhaps chiefly responsible for the failure of other promising treatments for American foulbrood; but even under the controlled conditions in the experimental apiary, recurrences of the disease in sulfa-treated colonies were observed. It is by no means certain that the six colonies listed in Table 2, though apparently free from disease, will not show evidence of it next year or the year after. Consequently, treatment with sulfa drugs may prove a long-term matter involving considerable time, labor, anxiety and uncertainty. It is in part for this reason that according to Eckert (2) "Most of the commercial beekeepers in California prefer to destroy the occasional diseased colony".

The masking and spread of the disease is one of the most serious considerations involved in the use of these drugs. The apparent disappearance of all visual signs of the disease may lead to careless handling on the part of the operator resulting in contamination of apiary equipment such as extractors, in the mixing of parts from infected hives with those from disease-free hives, in the mishandling of infected honey, and so on. For these reasons it has been suggested that sulfa treatment should be supervised by experienced personnel and that the infected treated colonies be isolated and placed in quarantine. The State of Florida (4) was the first to modify (not remove) its burning law to incorporate this treatment but only with the above provision.

It appears likely that sulfa drugs may play a definite though perhaps not a decisive role in the control of American foulbrood. It may be particularly useful as a preventive measure in areas where foulbrood abounds, for swarms of unknown origin and for package bees. The experimental work to date certainly warrants further extensive long-range trials by proper authorities.

ACKNOWLEDGMENT

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PREVENTION OF EARLY DECAY OF CUT POTATO SETS BY CHEMICAL TREATMENT[1]

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It is a general practice to plant potato sets without treating their freshly cut surfaces with a fungicide as a protection against soil-borne pathogens. Whole potato tubers are often disinfected before being cut in order to kill such superficial pathogens as *Actinomyces scabies* (Thaxter) Güssow, *Rhizoctonia Solani* Kühn, and certain *Fusarium* spp. However, this treatment gives the cut surfaces of the sets no direct protection against the common soil-borne pathogens *Erwinia carotovora* (Jones) Holland, *Fusarium coeruleum* (Lib.) Sacc., and *Pythium ultimum* (Trow.), all of which are known to occur in certain cultivated soils in Alberta and elsewhere. According to present evidence, *E. carotovora*, which is usually prevalent, often produces incipient rot in cut sets if they are stored too long in sacks prior to planting. This infection, and those that develop during the next 15 to 20 days after planting, may destroy the set or greatly reduce its propagative value, resulting in a weak plant. In an unfavourably wet soil such damage can be very severe.

According to Wollenweber (9), *F. coeruleum* will produce severe rot of potato tubers at temperatures ranging from 15° to 28° C. In the present study, this pathogen was found to destroy potato sets within a period of 21 days in soil held at a temperature of 61° F. *F. coeruleum* has been isolated in this laboratory many times from rotting potato sets collected from recently planted fields, as well as from rotting tubers obtained from commercial fields and from places of storage in Alberta. Although *Fusarium sambucinum* Fkl. f. 6 Wr. has often been isolated from certain lots of tubers rotting in storage in Alberta, this pathogen seems to be of little importance in the decay of cut sets in the field.

Under average field conditions in Alberta, *P. ultimum* appears less important than either *F. coeruleum* or *E. carotovora* in the rotting of freshly cut potato sets. However, Jones (4) states that in British Columbia *P. ultimum* causes extensive rotting of potato sets in certain heavy soils.

The present study was made to determine if some of the standard as well as certain of the new commercial fungicides when applied to the cut surface of potato sets before planting would protect them adequately against rot by common soil-inhabiting pathogens, particularly *F. coeruleum.*

MATERIALS AND METHODS

The various tests were made in untreated Edmonton black loam, the pH value of which is approximately 6.1. Although some of the trials were made in virgin soil, the majority were in soil from a field cultivated about 20 years, and in this paper it will be referred to as bin soil. The water content of the soil was maintained at approximately 23 per cent (dry), 30 per cent (optimum), or 34 per cent (wet) m.h.c., as required in

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the various experiments, and the soil temperature was maintained at 61° F. The soil in which the potato sets were placed for comparing the protective value of the various chemicals was artificially infested with soil-grown inoculum of *F. coeruleum*, and, in one trial, *P. ultimum*. The effectiveness of each chemical was tested on 30 potato sets of uniform shape and weight, cut from healthy tubers of the Warba variety with a vegetable baller. These sets were well mixed to provide, as far as possible, representative samples for experimental use. Unless otherwise stated, the sets were cut and immediately treated with the various chemicals as required, and buried at regular intervals in infested soil, where they remained for a period of 21 days. At the end of this period they were taken up, cut in halves at right angles to the periderm surface, and rated for the degree of rotting present.

The following chemicals and proprietary compounds were tested: mercuric chloride, yellow oxide of mercury, sulphur, calcium hydroxide, Semesan Bel (nitrophenol mercury + chlorophenol mercury), Dithane 14 (disodium ethylene bisdithiocarbamate), Roccal (10 per cent alkly-dimethyl benzyl ammonium chloride), Fermate (ferric dimethyl dithio-carbamate), Lunasan (ethyl mercury thiourea), Ceresan (5 per cent ethyl mercuric phosphate), and Spergon (tetra-chloro-para-benzoquinone). They were applied to the sets in the form of a solution, suspension, or as a dust, as indicated in Tables 1 and 2.

EXPERIMENTAL RESULTS

Experiments 1, 2 and 3

The purpose of Experiments 1, 2, and 3 was to ascertain whether or not freshly cut potato sets, when immersed in a solution of mercuric chloride (standard and acidified), or of Semesan Bel, are afforded a satisfactory degree of protection against attack by soil-borne *F. coeruleum;* and if any such protection is modified by the water content of the soil or by delay in the planting of the sets. Although the above mentioned chemical treatments are now used on whole tubers without serious injury to the vigour of the sprouts, very little is known regarding their effect on the natural healing processes of the freshly cut surface of a set, especially at lower temperatures in a soil which is unfavourably dry, or in one too wet. It is obvious that the potato set should have full protection immediately it is planted, since after about 15 to 20 days it will have established an independent plant.

According to data from the various tests in Experiments 1 and 3 (Table 1), the freshly cut sets treated with mercuric chloride or Semesan Bel were not satisfactorily protected against attack by *F. coeruleum*, although some benefit was usually evident. In the wet soil series of Experiment 2 (Table 1), there seems to have been practically no protection of the treated sets against a chance contamination of them or of the soil by *E. carotovora*. The untreated sets of the control in this soil series apparently escaped.

On the basis of the data for the untreated sets of the controls in all three experiments (Table 1), the sets in the dry soil series were more severely rotted than those in the wet soil, and particularly so in Experiment 3,

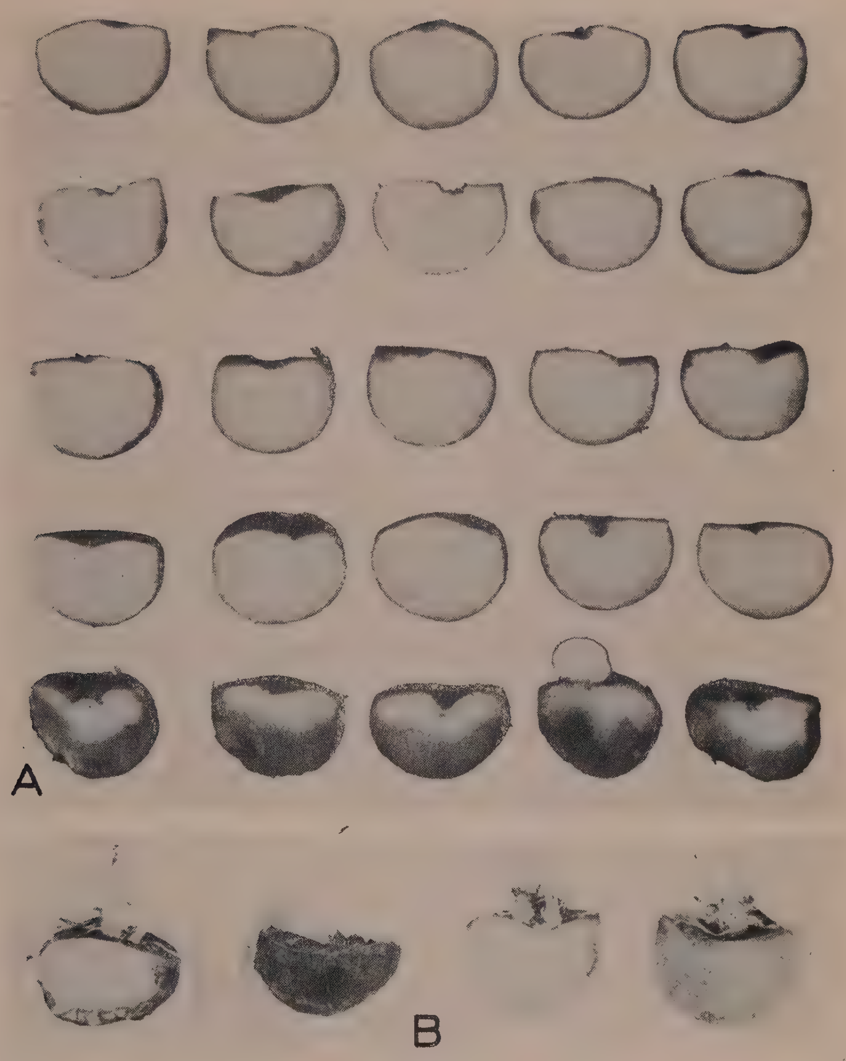

Figure 1

A. Chemical protection of freshly cut potato sets against rotting by *Fusarium coeruleum* in an artificially infested virgin soil. *Top row*, Spergon dust; *second row*, Spergon 1 per cent active chemical in 50 : 50 water-Aerosol solution; *third row*, Fermate dust; *fourth row*, not treated; *bottom row*, uninfested virgin soil, sets not treated.

B. Contrast in soundness of untreated sets in non-inoculated old cultivated soil (2 halves at left), and in virgin soil (2 halves at right).

TABLE 1.—DEVELOPMENT OF ROT IN TREATED CUT POTATO SETS IN NATURAL WET AND DRY SOIL[1], ARTIFICIALLY INFESTED WITH *Fusarium coeruleum*. THE SOIL WAS HELD AT 61° F. DURING THE 21-DAY INCUBATION PERIOD

Treatment	Time	Experiments							
		No. 1		No. 2		No. 3			
		Wet soil	Dry soil	Wet soil	Dry soil	Wet soil		Dry soil	
						A[2]	B[3]	A[2]	B[3]
	Min.	%	%	%	%	%	%	%	%
Mercuric chloride (1-1,000, plus 1 per cent hydrochloric acid)	5	5	28	86	46	38	28	30	25
	1	18	50	99	32	24	32	38	38
Mercuric chloride (1-1,000, not acidified)	5	16	6	62	24	21	24	42	44
	1	18	11	62	23	25	39	16	51
Semesan Bel (as recommended)	5	17	10	63	24	35	35	44	42
	1	15	21	43	31	7	10	41	27
Average of treatments	—	14.8	21	69	30	25	28	35.1	37.8
Sets not treated	—	40	62	19	38	37[4]	37[4]	63[4]	84[4]

[1] Approximately 34 per cent and 23 per cent m.h.c., respectively.
[2] Sets cut and planted immediately.
[3] Sets cut and held for 24 hours at 61° F.
[4] Based on 180 sets.

where the averages given are based on 180 sets, instead of on 30 as in Experiments 1 and 2. However, there was no significant difference in disease development when the sets were treated.

The data obtained on the effect of planting the treated cut sets immediately, or holding them at 61° F. for 24 hours, indicate no marked trend. However, in the untreated sets planted in dry soil, there appeared to be a difference of 21 per cent in favour of planting them immediately, but none when the water content of the soil was slightly above optimum.

From the evidence in Table 1, it appears that, in general, the treated cut sets were rotted slightly less severely by *F. coeruleum* than the untreated sets, especially when the latter were planted in a dry soil or held for some time before they were planted. However, it is clear that none of the chemical treatments employed gave sufficient protection to recommend their use in field practice.

Experiments 4, 5, 6 and 7

The results of the first three experiments (Table 1), indicated that further trials should be made with other chemicals. Four additional experiments were carried out at different periods. The water content of the soil in these experiments was maintained at approximately optimum, namely 30 per cent m.h.c. The data on the effects of the various chemicals tested on disease control and on sprout vigour are summarized in Table 2.

Under the conditions of these experiments, the sulphur, calcium hydroxide, Dithane 14, yellow oxide of mercury, and Roccal treatments gave very limited protection to the potato sets against attack by *F.*

TABLE 2.—SEVERITY OF ROTTING OF TREATED FRESHLY CUT POTATO SETS IN A SOIL ARTIFICIALLY INFESTED WITH *Fusarium coeruleum* OR *Pythium ultimum*

Treatment	*Pythium ultimum* Experiment No. 4 Bin soil	*Fusarium coeruleum* Experiment No. 4 Bin soil	Experiment No. 5 Bin soil	Experiment No. 6 Virgin soil	Experiment No. 7 Virgin soil	Vigour of Sprouts
	%	%	%	%	%	
Sulphur (dust)	89	91	—	—	—	Average
Fermate (dust)	0	0	0	—	0	Average
Fermate, 1 per cent	32	87	—	—	—	Average
Dithane, 0.4 per cent	40	68	—	—	—	Average
[1]Yellow oxide of mercury, 0.4 per cent	—	33	27	—	—	Average
Calcium hydroxide (dust)	—	—	38	—	—	Average
[2]Roccal, 1 per cent	—	—	17	—	—	Average
[2]Lunasan, 1 per cent	—	—	0	—	—	Stunted
Lunasan, 0.5 per cent	—	—	1	—	—	Weak
[2]Ceresan, 1 per cent	0	0	0	—	—	Dormant
Ceresan, 0.5 per cent	—	—	0	—	—	Dormant
[2]Spergon (dust)	—	—	0	0	0	Strong
Spergon, 2 per cent	—	—	—	0	0	Strong
Spergon, 1.5 per cent	—	—	—	0	0	Strong
Spergon, 1 per cent	—	—	—	1	Trace	Strong
Untreated sets	67	80	53	82	26	Average
Uninfested soil, untreated sets	—	—	10	0	0	Average

[1] Optimum water content 30 per cent m.h.c., and temperature 61° F.
[2] Dip treatments showing percentage of active ingredients per litre of 50 : 50 Aerosol—water (except Roccal).

coeruleum. On the other hand, Fermate applied as a dust, Lunasan and Ceresan applied as dip treatments, and Spergon applied either in dust form or as a 50 : 50 water-Aerosol solution containing as low as 1 per cent active ingredients gave practically 100 per cent protection to the sets (Figure 1A).

With reference to the soil series infested with *P. ultimum* (Experiment 4, Table 2), sulphur was useless; the Dithane and Fermate dip treatments were only partially effective; but Fermate applied as a dust, and Ceresan as a dip treatment, afforded the sets apparently complete protection. Spergon was not included in this series.

With reference to the effect of the various chemicals used in these experiments (Tables 1 and 2) on the vigour of the sprout of the treated set, certain important differences were observed. The sprouts from sets treated with Spergon seemed uniformly above normal in vigour in all tests. In contrast, sprouts were not produced by the sets treated with Ceresan, and they were stunted or weak in the Lunasan treatment. None of the remaining chemical treatments consistently reduced sprout vigour, although occasionally both Semesan Bel and mercuric chloride seemed to be detrimental.

Finally, attention is directed to the typical contrast (Figure 1B) in soundness and colour of the untreated sets incubated in the "uninoculated" control series of the virgin and cultivated soils used (Table 2, Experiments 5 and 6). Numerous spores of *Fusarium* spp., evidence of other fungi, and

many starch grains were found in the decaying tissue of the sets. Consequently, it is suggested that unprotected sets in an infested soil could be a fruitful source of inoculum potentially dangerous to the new crop of potato tubers during harvesting operations and later in storage.

DISCUSSION

Cunningham and Reinking (3), in their studies of Fusarium seed-piece decay of potato on Long Island, U.S.A., feel that the treatment of whole tubers is best suited to farm practice because they can be treated when convenient and stored until cutting time. However, they warn against contamination of the freshly cut sets and stress that, if these must be held any length of time before being planted, they should be stored under conditions favourable for rapid healing of their cut surfaces. Under average farm conditions, the freshly cut sets are very likely to be contaminated with pathogenic fungi and bacteria. In Alberta, the cut sets are often held in storage at temperatures around 60° F., or lower, during several days. Even if they are planted the same day as cut, the temperature of the soil at this time of the year is more likely to be less than 60° F. than higher.

According to Priestley and Woffenden (5), wound periderm in potato tubers may not appear until 12 to 15 days at 61° F. Also, several investigators (1, 2, 5) have found that the healing processes, namely suberization and wound periderm formation, develop more slowly in certain varieties than in others. In a separate study, the results of which will be reported later, wound periderm appeared in about 10 days in cut potato sets buried in moist soil at 61° F., whereas the blocking-off process of the cut surface apparently was not well advanced before the end of three days.

Weiss, Lauritzen and Brierley (8) concluded from their comprehensive infection studies that at 61° F. the two or three layers of cells immediately below the cut surface which become suberized (2) required fully three days to heal well enough to resist penetration by *F. coeruleum* effectively. Successful fungal infection during this vulnerable period could arrest the natural healing processes.

The experimental data presented in Table 2 show that at 61° F. freshly cut potato sets, planted immediately in a natural soil of optimum water content and artificially infested with *F. coeruleum*, were very severely rotted (Figure 1A) within a period of only 21 days. This could happen in the field and be accentuated by bacteria causing soft rot. It was also shown in Table 2 and Figure 1A that, under the same conditions, the sets remained sound when their cut surfaces were treated with Fermate applied as a dust, Lunasan and Ceresan applied as dip treatments, and Spergon applied either as a dust or a dip. The degree of control obtained from Semesan Bel, Dithane, yellow oxide of mercury, mercuric chloride, sulphur, and calcium hydroxide was inadequate for the purpose intended. Although none of the chemicals in this latter group obviously reduced the vigour of the sprouts, those containing mercury were suspected of lowering sprout vigour. The sprouts were quite weak to stunted when the sets were treated with Lunasan, but wholly suppressed by Ceresan. They seemed uniformly stronger than normal on the sets treated with Spergon.

The foregoing data seem to indicate that, under field conditions, highly beneficial results of commercial importance may be obtained by treating freshly cut potato sets with certain chemicals.

It may be asked if the treatments suitable for controlling rot by *Fusarium* spp. in cut sets would be as effective in reducing the development of common scab and stem canker as those now recommended for whole tubers. The answer is not available from the data of the present study, but could be obtained by making controlled tests of promising chemicals in the laboratory. Excepting when the surface of treated whole tubers is heavily infested with scurf or sclerotia of virulent races of *R. Solani*, the detection of a difference between the two methods in disease control would be difficult in field culture (6, 7).

SUMMARY

The value of various chemicals for protecting freshly cut potato sets, planted in field soil, against attack by *Fusarium coeruleum* was compared in controlled laboratory experiments. The soil was artificially infested with the pathogen, maintained at a temperature of 61° F. and a water content (30 per cent m.h.c.) about optimum.

Untreated sets were severely rotted within 21 days, but when the sets were dusted with Fermate, or when Semesan or Lunasan were applied as dip treatments, or Spergon used as a dust or applied as a dip (1 per cent active ingredient), they remained sound. Sets treated with Semesan Bel, Dithane, yellow oxide of mercury, mercuric chloride, sulphur, or calcium hydroxide were afforded inadequate protection.

Apparently the pathogen rotted untreated sets more when the soil was rather dry (23 per cent m.h.c.) than when fairly wet (34 per cent m.h.c.).

Excepting Ceresan and Lunasan, the chemicals used were not obviously detrimental to sprout vigour. Ceresan suppressed the sprouts and Lunasan stunted them. Spergon seemed to increase sprout vigour.

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A NOTE ON THE CAUSE OF BRITTLE DWARF OF WHEAT

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The name Brittle Dwarf was suggested by Fraser *et al.* (2) in 1932, for a disease of winter and spring wheats characterized by a stunting and extreme brittleness of the culms, usually accompanied by a proliferation of the shoots and yellow mottling and streaking of the leaves (Figures 1 to 4). Other occasional features were malformed heads, distorted peduncles, and twisted terminal leaves. Aphids were usually associated with the diseased plants and were frequently present in large numbers under the leaf sheaths and in twisted leaves. The trouble appeared to spread from an infection source; it occurred both in patches and on isolated plants. The plots of spring wheat affected with brittle dwarf were adjacent to the affected winter wheat plots, though this is not mentioned in the first communication (2). Seed transmission was not obtained experimentally. H. H. McKinney, U.S. Dept. of Agriculture, to whom specimens were sent, concluded that it was not the same as the mosaic of wheat (7) occurring east of the Mississippi River.

Since 1932 the disease has been reported several times on wheat in various parts of Saskatchewan, being usually found at the edges of fields. In one field, 25 per cent of the culms were affected in a patch 50 feet in diameter (4). It was first recorded on barley by W. G. Sallans and R. J. Ledingham (3) in 1933, in an experimental plot in which 7 per cent of the plants were affected. Records of the disease have also been made by entomologists (1). Renewed interest in the disease was recently aroused by Simmonds (5) in 1947. Affected wheat and barley plants were observed scattered through his experimental plots. A. P. Arnason of the Dominion Laboratory of Entomology pointed out to him the similarity of injury on nearby crested wheat grass (*Agropyron cristatum* (L.) Gaertn.) caused by aphids, and that on the wheat and barley, and also drew to his attention a similar trouble described on winter wheat by Parker (8) in 1916, in Montana, as being caused by the western wheat aphis (*Brachycolus tritici* Gill.). Simmonds suggested that the serious nature of the outbreak on one of our common forage grasses pointed to the necessity of both entomologists and plant pathologists studying the etiology of the disease. The increasing production of winter wheat in southern Alberta and southwestern Saskatchewan adds further weight to this contention.

No indication of the presence of nematodes in diseased plants could be found so that any similarity to the symptoms of the disease of wheat caused by *Tylenchus tritici* (S.) Bast. (6) was merely fortuitous. No cell inclusions were observed in mottled or streaked leaves. Experiments had shown that the trouble was not seed-borne. These two facts ruled out mosaic or rosette (7) as being concerned. A bacterial disease associated with some affected plants in 1925 and 1928 was believed to be black chaff (2). It was later found that these plants were hybrid material very

[1] Contribution from the Laboratory of Plant Pathology, University of Saskatchewan, Saskatoon, Sask. with financial assistance from the Saskatchewan Agricultural Research Foundation.

[2] Professor of Plant Pathology.

susceptible to black chaff (*Xanthomonas translucens* f. sp. *undulosa* (E.F. Sm., L. R. Jones, and Reddy) Hagb.). No evidence remained suggesting a pathogenic relationship to the disease. The main evidence in support of the virus hypothesis was that affected plants were sometimes found on which aphids were very few or entirely absent. Final speculation as to the cause was twofold: Was the injury due wholly to aphids, or were they also serving as vectors of a virus?

EXPERIMENTAL

Preliminary experiments were conducted in 1931 and again in the autumn of 1947[1] to test the transmissibility of brittle dwarf by the wheat aphis. In the earlier tests aphids were taken from affected wheat plants and allowed to feed on healthy wheat seedlings for one or two days; in the later experiments the aphids[2] were taken from both affected wheat and crested wheat grass plants. No evidence of insect transmission of the disease was obtained.

In late June, 1948, severe brittle dwarf developed on spring-sown perennial wheat plants (*Agropyron elongatum* Host × *Triticum vulgare* Vill. var. Chinese) adjoining pots of overwintered perennial wheat (Figures 1 and 3). Aphids from affected plants were used to inoculate healthy wheat seedlings.

Pots

Thatcher wheat was sown in 6-in. pots, three seeds to each pot, and when the seedlings were 4 in. high an experiment comprising three series as outlined below was set up. Three such experiments were conducted during July and August; the first began on July 14 and the others at about weekly intervals thereafter.

Series I.—Aphids were allowed to feed on one pot of seedlings for 24 hours, and on another pot for 48 hours. After removal of the aphids, the pots were then placed in a fine-meshed wire cage (28 mesh to the inch) and sprayed with nicotine sulphate solution. On the same evening, the plants were fumigated with Nicofume.

Series II.—Two pots of seedlings were similarly inoculated with aphids and placed under screens in an adjoining compartment of the greenhouse. The aphids were reproducing parthenogenetically and in the course of a few days the plants were heavily infested.

Series III.—Two pots of seedlings were kept free of aphids.

Seven weeks after the first experiment was set up, the plants in Series I and III had headed out, averaged 20 to 21 inches in height and showed no signs of brittleness. Those in Series II were about 7 inches high, brittle and severely mottled and streaked. Symptoms as severe as these are sometimes encountered in the field (Figure 3).

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[1] The 1947 experiments were conducted by W. M. Dion, to whom thanks are due for permission to include her results.

[2] A. P. Arnason kindly identified these as the western wheat aphis (*Brachycolus tritici* Gill.).

FIGURE 1. A typical portion of a row of spring-sown perennial wheat affected with brittle dwarf. The "escaped" culms may be slightly brittle.

FIGURE 2. Mottled and streaked leaves, a distorted head and a crinkled and twisted leaf.

FIGURE 3. Two plants with severe symptoms—stunting, conspicuous yellow mottling, extreme brittleness, and empty heads.

FIGURE 4. Spring wheat plants affected with brittle dwarf.

The results of the two other similar experiments were virtually the same. In Series II, care had to be exercised so as not to allow the infestation to become too heavy; otherwise the plants would be killed before becoming brittle.

The foregoing experimental results give no indication that a virus is associated with the disease. They point strongly to the conclusion that the so-called brittle dwarf disease of wheat, barley, crested wheat grass and other grasses is caused by the western wheat aphis.

Parker (8) has mentioned the severe damage which can be caused by this aphis and our observations in Saskatchewan bear this out in limited areas. He found, however, that during the hottest summer weather, coccinellid and hymenopterous parasites greatly reduce the numbers of aphids. This may explain why only localized outbreaks have occurred in Saskatchewan in the past. Practical recommendations for the control of this aphis may be found in Parker's paper.

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CONCERNING THE MOVEMENT OF THE BULB EELWORM, *DITYLENCHUS DIPSACI* (KUHN) FILIPJEV, IN NARCISSUS BULBS[1]

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In his description of the eelworm disease of narcissus, caused by the bulb eelworm *Ditylenchus dipsaci* (Kuhn) Filipjev, Goodey (1) states that the worms wander down into the tissue of the particular bulb scale which they first enter, and there produce a brown ring of diseased tissue, and that, as the disease advances, more and more of the fleshy scales become invaded until finally the whole bulb may be involved. However, he does not explain how the disease spreads to scales other than those first entered by the worms. Stillinger (2) states that the worms work between the cells, causing the disintegration of the middle lamella, and that the affected scales turn yellow or brown. He notes that infection may extend laterally, but he found that it advances more rapidly towards the base of the bulb and does not extend across from one scale to another. He concludes that, within the bulb, the spread of infection from scale to scale takes place by movement of the worms to the base and from the base to uninfected scales.

From these accounts, it may be inferred that the worms occur in the brown scales only, these scales forming the brown ring symptom typical of an eelworm infection. However, evidence has been secured that the bulb eelworm may also occur in the white scales lying between brown scales.

A lot of narcissus bulbs, variety Laurens Koster, was collected from the stock of a British Columbia bulb-grower on August 10, 1948. Many of these bulbs were soft as a result of eelworm infection, and a fair proportion of them carried eelworm-wool. Dissection of these bulbs showed that the sheaths of many uncoloured scales could be readily separated because of the disintegration of the middle lamella of the cells of the scales. Scrapings from the inner surface of these scales disclosed the presence of the bulb eelworm in all stages of development. Longitudinal strips of uncoloured scales were divided into quarters for examination. The eelworms found in the basal quarter were mostly pre-adults, but those in the apical quarter were mostly eggs and young larvae. Furthermore, a number of bulbs were cut across in the neck region, and the white scales lying between brown scales were examined. Eggs and young larvae and a small proportion of adults and pre-adults were frequently encountered.

A study was also made of a lot of narcissus bulbs, variety King Alfred. These bulbs arrived in British Columbia from Great Britain and were inspected on October 29, 1948. A small percentage of the bulbs was found infected with the bulb eelworm. Examination of the infected bulbs disclosed that the worms had moved through the initially infected scales into the basal plate, and from there had spread laterally to several consecutive scales. The brown discoloration was present only in the basal portion of the

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infected scales. The scales were divided horizontally into quarter-sections for examination. The eelworm population found in the brown basal section was dense, but in the next two non-coloured sections it was relatively sparse. No eelworms were found in the apical section. The embryo leaves which had grown almost level with the crown of the bulb were also found to be infected. Infection was confined to the lower half, and a slight brown discoloration had developed.

The data secured from the English bulbs support the conclusion of Stillinger (2) that the spread of infection from scale to scale takes place by way of the basal plate, and that the infected scales turn brown. In this instance, the brown discoloration was only in the basal quarter-section of the scale where the eelworm population was more dense than in the uncoloured sections. There appears to be a connection between the age of infection and the extent of discoloration. This conclusion is supported by the data from the two lots of bulbs under study. The eelworms in the British Columbia bulbs were distributed from the base to the apex of the infected scales, but the scales were not discoloured when these observations were made on August 10. On the other hand, the eelworms in the English bulbs were found in the basal section and also the next two sections of the infected scales. They were not found in the apical section. The basal section was the only one that was discoloured. These observations were made on October 29. It can be inferred that the eelworms moved more rapidly through the British Columbia bulbs than through the English bulbs, and consequently there was no discoloration in the British Columbia bulbs.

The evidence of rapid movement of the worms through the British Columbia bulbs supports evidence that eelworm-wool appears earlier and more frequently in local bulbs than in bulbs from England and the United States. In 1934, correspondence with the United States Division of Nematology indicated that eelworm-wool was not recorded in Washington State before early September or in New York State before late September. At that time, Hastings (3) had already reported that eelworm-wool was found in British Columbia bulbs on July 26. On the other hand, eelworm-wool was found on a lot of imported English bulbs on September 29. Recently (April, 1948) a letter from a nematode authority in Washington State stated that eelworm-wool does not form readily in that State in most years. The environmental conditions in British Columbia are apparently more favourable than in the United States, and possibly also in England, for the development of eelworm populations.

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